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Peltonen, Leena

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Practical guidelines for the characterization and quality control of pure drug nanoparticles and nano-cocrystals in the pharmaceutical industry

Leena Peltonen

Division of Pharmaceutical Chemistry and Technology, Drug Research Program, Faculty of Pharmacy, University of Helsinki, Viikinkaari 5 E, P.O. Box 56, FI-00014 Helsinki, Finland

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ABSTRACT

The number of poorly soluble drug candidates is increasing, and this is also seen in the research interest towards drug nanoparticles and (nano-)cocrystals; improved solubility is the most important application of these nano-systems. In order to confirm the functionality of these nanoparticles throughout their lifecycle, repeatability of the formulation processes, functional performance of the formed systems in pre-determined way and system stability, a thorough physicochemical understanding with the aid of necessary analytical techniques is needed. Even very minor deviations in for example particle size or size deviation in nanoscale can alter the product bioavailability, and the effect is even more dramatic with the smallest particle size fractions. Also, small particle size sets special requirements for the analytical techniques. In this review most important physicochemical properties of drug nanocrystals and nano-cocrystals are presented, suitable analytical techniques, their pros and cons, are described with the extra input on practical point of view.

1. Introduction

Drug nanocrystals are pure solid drug nanoparticles, which are covered by a stabilizer layer [1–4]. In rare cases, no stabilizer is needed [5]. But, mostly a layer of polymer(s) and/or surfactant(s) is needed for stabilizing nanoparticles against particle aggregation. Stabilizer layer can be based on only one material, but often also a mixture for example of one polymer and one surfactant is used [6]. Functionality of drug nanocrystals can be reached *via* adding some functional/linking groups on the stabilizer layer [7–9]. Often nanocrystals are referred to solid micelles.

Cocrystals are multicomponent solids, where the crystal contains two or more molecular components, in pharmaceutics, active pharmaceutical agent (API) and cocrystal former, coformer [10–14]. In cocrystals different components have well-defined stoichiometric structure in a single homogenous crystal phase. Cocrystals are formed *via* different types of molecular interactions: hydrogen bonds, π - π stacking and van der Waals forces (Fig. 1). In cocrystals, drug and coformer interact nonionically, which separates them from salts [14]. Hence, cocrystals provide another possibility for poorly soluble drugs for improved solubility: they often have no ionic functional groups that are demanded for salt formation. Often cocrystals are referred to solvates/hydrates, where typically nonvolatile coformer is not a solvent or water. Pharmaceutical cocrystals have been studied for years for improved solubility, and nano-cocrystals are just cocrystals in nanometer scale [15,16].

Besides solubility, cocrystallization can improve also other physicochemical properties of the drug: chemical stability, dissolution rate, mechanical properties (process ability) of the drug, hygroscopicity, compressibility and flowability [17]. For example, drug melting point can be changed *via* cocrystal formation [18]. Typically, cocrystalline drug has a melting point in between of the melting point of the pure drug and the coformer. The situation can also be different, and in that case, the high melting point indicates poor solubility. When the cocrystals are made in nanoscale, *i.e.* nano-cocrystals, for example solubility and dissolution rate can be improved even further. Improvements are not only due to the cocrystals structure, but also due to the nanonized size scale, which means increased surface area for dissolution.

If drug nanocrystals and nano-cocrystals are compared to other molecular level techniques utilized for improved solubility, they differ from salts in that they do not require any ionic interactions. This means that non-ionic materials can be used. To amorphous systems the difference is that crystal form is thermodynamically stable, but the stability challenge is in aggregation tendency of small particles. With both the drug nanocrystals and drug nano-cocrystals, the solubility enhancement can in best cases be in the same level as with amorphous systems [19,20]. Cocrystal solubility enhancement can be tailored to a certain level by solution pH changes, solubilizing agent concentration or

E-mail address: leena.peltonen@helsinki.fi.

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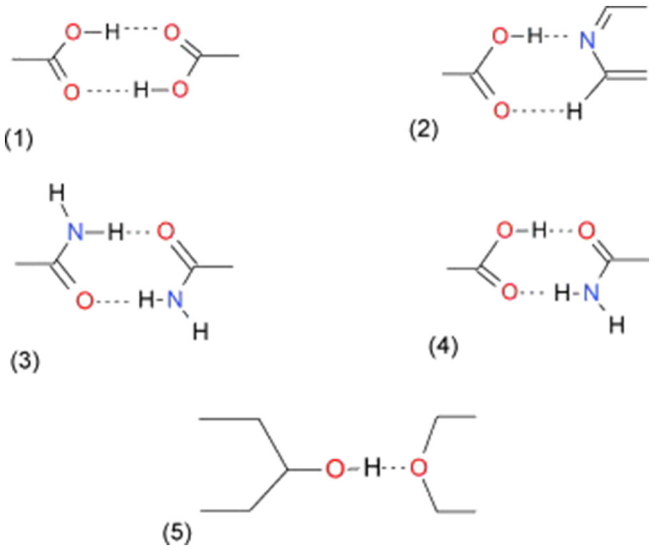

Fig. 1. Hydrogen bonding groups typically utilized in formation of pharmaceutical cocrystals: (1) carboxylic acid homosynthon; (2) carboxylic acid – pyridine heterosynthon; (3) amide homodimer; (4) carboxylic – amide heterosynthon; (5) alcohol - ether heterosynthon. Reprinted from [10] with permission from Elsevier.

coformer concentration (Fig. 2) [11, 21–23]. More precise description of cocrystal formation can be found for example from [11,24,25].

Cocrystal formation is utilized mainly for improved solubility and hence drug is often hydrophobic, but the coformer hydrophilic in nature. Solubility enhancement is correlated with coformer solubility, because coformers decrease the solvation barrier of the whole cocrystal system [11].

Typically drug nanocrystals and nano-cocrystals dissolve almost immediately, when they get into contact with the fluids *in vivo*. However, for example for intravenous (i.v.) administration [26] or implants, when the dissolution and drug release is not necessarily immediate, the size, shape, surface coating/charge, and morphology are important product properties. Sigfridsson et al. [26] studied *in vivo* distribution of drug nanocrystals in mice after i.v. administration. Nanocrystals were produced by milling with different surface materials: DSPE-PEG2000 (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(poly(ethylene glycol)) PEG-ylated and Pluronic F127, both with or without polyvinylpyrrolidone K30 (PVP)/Aerosol. DSPE-PEG2000 PEG-ylated particles had prolonged circulation time (particle elimination half-life 9 min) compared to DSPE-PEG2000/PVP/Aerosol formulation (half-life 3 min), while both the Pluronic F127 formulations possessed similar half-lives (9 min). In the study, dissolved drug were accumulated in hepatocytes. But, undissolved particles were accumulated in the liver sinusoidal endothelial cells and Kupffer cells. Further, DSPE-PEG2000/PVP/Aerosol stabilized particles were accumulated more in the liver, compared to Pluronic F127/PVP/Aerosol stabilized particles.

In the drug nanocrystal and nano-cocrystal applications it is important to recognize, if the particles are meant to be dissolved immediately or if the aim is controlled drug release from solid particles: this affects the critical quality attributes, CQAs, and desired particle properties of the end product [27–29]. In this review, most important physicochemical properties, based on CQAs, of drug nanocrystals and nano-cocrystals are presented with practical case studies. Physical stability (stable particle size and solid state form), solubility behavior, and drug release testing are discussed in detail. Suitable analytical techniques, their pros and cons, are described from the practical point of view. Main emphasis is put on the characterization of fast dissolving systems, but controlled release systems are also shortly discussed.

2. Critical quality attributes of drug nanocrystals and nano-cocrystals

Depending on the target product profile, quality attributes of the drug nanocrystals and nano-cocrystals may differ. Shortly, depending on the *in vivo* faith of the nanoparticles, they can be divided into two classes: (1) nanoparticles meant to be taken up by the cells as solid particles, and (2) fast dissolving nanoparticles where fast dissolution is followed by absorption of dissolved drug.

2.1. Solid nanoparticles in vivo

As already discussed, nanoparticles can have different *in vivo* pharmacokinetic profiles, biodistributions and therapeutic efficacy due to the size, shape, surface charge and surface hydrophobicity properties [29]. These properties are very important, if the nanosystems are not intended to be dissolved immediately. If the particles are taken up by the cells as solid particles (before particle dissolution, Fig. 3) particle shape has been shown to affect cellular uptake kinetics and mechanisms, level of uptake, and intracellular distribution, and, hence, cytotoxicity [30]. Anticancer agents reached improved therapeutic efficacy, when non-spherical particles were used [30]. Nanoparticle shape (spherical *vs.* rod shaped) caused different membrane bending energies during endocytosis, which affected cell uptake [31]. Xie et al. [32] studied the shape effect on cellular internalization of gold nanoparticles with RAW264.7 cells. Studied shapes were stars, rods and triangles. In the study, the cellular uptake was highest with triangle shaped nanoparticles, and lowest with star shaped systems.

Particles are mostly internalized into the cells *via* pinocytosis (micropinocytosis or macropinocytosis) or phagocytosis [34,35]. When detecting only the particle size, Swanson and Watts [36] found out in their study that particle sizes above 200 nm were internalized by phagocytosis or micropinocytosis, while particles below 200 nm were taken up in all the cell types *via* micropinocytosis (either clathrin mediated, caveolae or lipid raft mediated or clathrin or caveolae independent mechanisms) [37]. However, besides particle size, other factors like particle shape and surface properties, as well as studied cell line, are affecting uptake mechanisms. In the following section, case studies

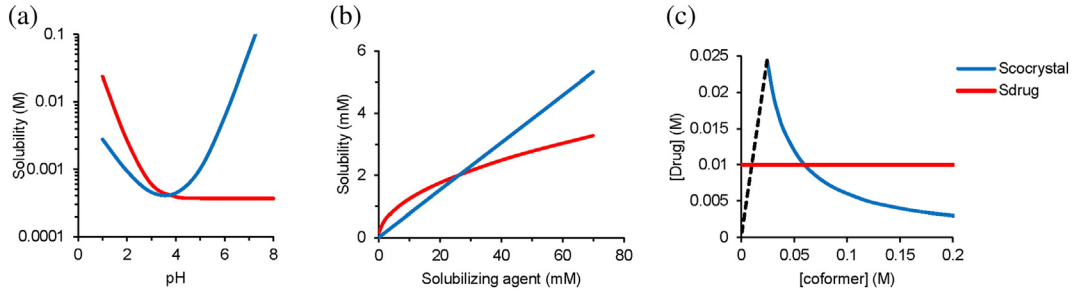

Fig. 2. Solubility of cocrystals. Solubility as a function of (a) pH, (b) concentration of solubilizing agent, and (c) concentration of coformer. Dashed line corresponds stoichiometric (1:1) concentrations of drug and coformer. Reprinted from [11] with permission from Elsevier.

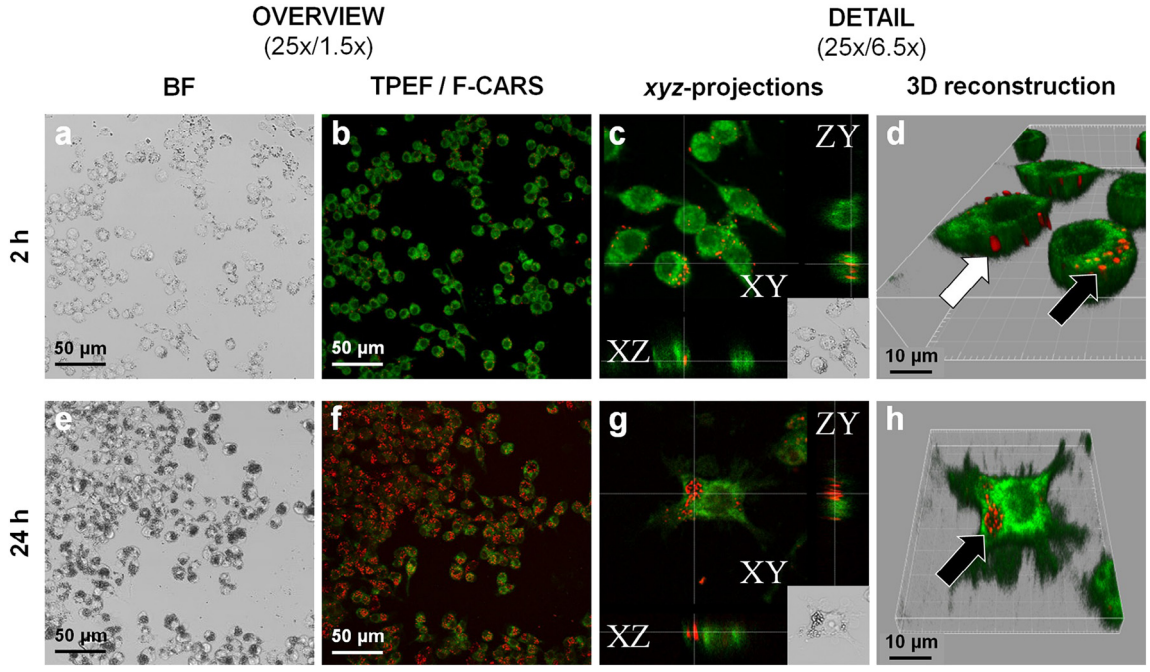

Fig. 3. Cell uptake of paliperidone palmitate drug nano−/microcrystals in RAW 264.7 macrophages by CARS (Coherent Anti-Stokes Raman Scattering) imaging. Figure (a) and (e): low and high magnification bright field images; figures (b) and (f): F-CARS (forward-detected CARS; red)/TPEF (two-photon excitation fluorescence; green) merged micrographs; figures (c) and (g): orthogonal projections of z-stacked F-CARS/TPEF overlays showing intracellular solid nano−/microcrystals; and figures (d) and (h): 3-D reconstructions of the z-stacked F-CARS/TPEF overlays. The arrows show nano−/microcrystals adsorbed onto the cell surface (white arrow) and phagocytosed (black arrows). Incubation time with nano−/microcrystals 2 h (a–d) and 24 h (e–h). Reprinted from [33] with permission from Elsevier. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

related to cell uptake of nanoparticles with different size, surface properties and shape, are presented, but the reader should be aware of that cell uptake is complex process with many variables and more systematic studies are needed in that area.

When cell uptake of gold nanoparticles with particle size of 80-90 nm were studied, the uptake was following: triangle shaped nanoparticles were taken up *via* clathrin mediated mechanisms and rod shaped *via* caveolae and clathrin mediated mechanisms [32]. Clathrin mediated endocytosis could be followed by exocytosis where part of the nanoparticles were returned to extracellular area [38]. Caveolae mediated cell uptake can be either *via* caveosome pathway, where part of the nanomaterial is removed from the endoplasmic reticulum/Golgi body, or *via* endosomal route, where nanomaterial may be exocytosed. Accordingly, in the caveolae mediated route there were different exocytosis pathways, which was suggested to be the reason behind the lower level of cell uptake with rod shaped nanoparticles.

Uptake studies of negatively charged 10-hydroxycamptothecin (approximated particle size 130 nm) with 4T1 cancer cell line showed that internalization was mainly mediated by clathrin dependent endocytosis [5]. Nanoparticle shape were also shown to enhance drug therapy in lung and brain endothelium [39] and in vascular targeting [40] combined with lower level of liver and spleen accumulation [41]. Besides the shape, surface layer properties affect the internalization: Poloxamers are well known to interact with the cell membrane, and Poloxamer 407 stabilized nanosystems were efficiently internalized by cancer cells *in vitro* [42,43].

When considering effect of surface charge on cell interactions, phagocytic cells uptake more readily negatively charged particles than neutral ones, and negatively charged particles are cleared faster from the blood [44]. On the other hand, positively charged nanosystems can interact with negatively charged serum proteins [45] and also with negatively charged eukaryotic cell membranes [46]. Cancer cell membranes can overexpress negatively charged phospholipids [47]. The cell uptake of 10-hydroxycamphothecin nanocrystals (zeta potential −27 mV) into 4 T1 cancer cells was mainly mediated by clathrin-dependent endocytosis [5].

Drug nanocrystals/nano-cocrystals can be utilized for controlled drug delivery [8,48–52]. For example, in oral delivery, drug nanocrystals were imbedded into matrix structures for sustained drug release [8], and *in vivo* passive accumulation of drug nanocrystals was reached *via* mononuclear phagocytic system (MPS) cells, which led to passive accumulation of particles in MPS-rich organs, like liver, spleen and lung [49]. Effect of particle size on drug release profile was shown with two differently sized oridonin nanosuspensions: formulation A with approximated particle size of 100 nm and formulation B 900 nm [51]. With 100 nm nanocrystals, *in vitro* dissolution was completed in 10 min, while with 900 nm nanocrystals only 85.2% of drug was dissolved after 2 h time. Tissue distribution after intravenous administration showed that pharmacokinetic profile and biodistribution of drug from 100 nm oridonin nanosuspension was similar to solution. However, 900 nm nanosuspension showed higher drug uptake in the reticuloendothelial system (RES) organs and had markedly different pharmacokinetic profile compared to 100 nm particles. An example of long-acting intramuscularly injected drug nano−/microcrystal system is Xeplion® with paliperidone palmitate as an active agent, which has therapeutic drug release profile up to several months [52,53].

2.2. Fast dissolving nanosystems

Above mentioned examples do emphasize the importance of exact knowledge of particle size, size deviation, shape, composition and morphological information, which affect on cell uptake. With fast dissolving nanosystems, the aim is that particle dissolution *in vivo* is immediate, which is followed by efficient drug permeation (of dissolved drug); most of the drugs are belonging to Biopharmaceutics Classification System (BCS) class II, where the poor solubility is the rate limiting step for permeation [7,54,55]. In these systems, particle shape or size does not affect cell uptake (drug permeates in dissolved form), but do affect for example dissolution rate, solubility, and formulation related factors like aggregation tendency and physical stability of the system. Accordingly, particle size should be tailored to be optimum according to the

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administration route and formulation requirements, taken into account in the Quality Target Product Profile (QTPP).

2.3. Critical quality attributes, CQAs, for drug nanosystems

Critical Quality Attributes, CQAs are selected based on QTPP. For example particle size, shape, solid state properties or cocrystal structure, stability or solubility can be CQAs [30,56]. Stability, and especially physical stability, is important CQA. In the case of drug nanocrystals and nano-cocrystals, physical stability can be divided to i) constant particle size and particle size deviation and ii) unaltered/controlled solid state. In the case of drug nano-cocrystals solid state characterization is required also to confirm the formation of certain cocrystal structure: that nonionically interacting drug(s) and coformer(s) co-exist in the unit cell [14].

Other aspects of stability issues of cocrystals are stability against humidity, thermal stability, chemical stability and solution stability (ability to stay in solution). With cocrystals higher amount of water can state stability problems [18]. Solution stability is important issue in further drug development with both the drug nanocrystals and nano-cocrystals, because the apparent solubility of both the systems are higher than thermodynamic solubility of the drug, *i.e.* the solution is in supersaturated state. Accordingly, uncontrolled crystallization/precipitation may follow after the fast dissolution, and this can hinder permeation [50]. Drug release tests for nano-cocrystals are required in order to show that drug is dissociated from the co-crystal structure and dissolves as an original drug.

3. Characterization and quality control of drug nanocrystals and nano-cocrystals

The quality and analysis of nanosystems is much more complicated as compared to bulk material or even to microparticle systems [27]. In nanoscale many physical properties are different from corresponding bulk values, for example, in submicron area, apparent solubility can be considerably higher than corresponding saturated solubility value of bulk material [28]. Nanoparticles are inherently unstable and have high tendency for aggregation. Also, the role of sampling is crucial for nanoparticulate systems, as well as sample handling. Care must be taken that sampling is representative for the whole batch. In particle sizing, for example, for very polydisperse samples incorrect particle size information may be resulted after the sedimentation of larger particles, when only the smaller particles are analyzed, or, other way around, the smallest particles are dissolved during the sample dilution. Most relevant characterization techniques for nanosystems are listed in Table 1.

Table 1
Properties of nanosystems and most relevant characterization techniques.

Quality attribute	Method	Comments
Particle size	Non imaging: light scattering based techniques, like dynamic light scattering (DLS)	Fast, repeatable, shape information not available, careful sample preparation and sampling, measurement from suspension, with new sample advised to be combined with some imaging technique
	Imaging based techniques: scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM)	Time consuming, measurement from dried sample, also shape and morphological information available, only part of the sample is analyzed
Particle shape, morphology	Imaging: SEM, TEM, AFM	Drying and in SEM sputtering of the sample may change properties
Surface properties	zeta potential	Zeta potential dependent on liquid phase properties
Solid state properties	Spectroscopy: infrared (IR), Raman, solid state nuclear magnetic resonance (SSNMR)	Solid state characterization, interaction studies, limited information of chemical structures
	X-ray diffraction (XRD)	Solid state characterization
	Thermal analysis Differential scanning calorimetry (DSC), thermogravimetric analysis (TGA)	Solid state characterization, interaction studies, some information for chemical structure determination
Chemical structure and degradation	Liquid chromatography like high performance liquid chromatography (HPLC)	Chemical stability and degradation
Dissolution and solubility testing	Intrinsic dissolution, dissolution/drug release rate, apparent solubility	Method selection based on the aims: quality control or research purposes

3.1. Particle size, shape and morphology

Particle size, size deviation, shape and morphology are important indicators for small particle systems. The size, size deviation as well as shape and morphology are related to stability, physicochemical performance like dissolution and solubility as well as cell uptake and final fate *in vivo* of nanosized materials, as already described earlier [57,58]. Drug nanocrystals can be stabilized against aggregation with the aid of stabilizer layer [59]. In general, when thinking about the effect of particle size and size deviation on stabilization, the smaller the particle size, the more the particles tend to aggregate. Similarly, more heterogeneous particles in size (larger size deviation) are also more unstable.

When selecting the measurement technique, it is important to take into account the measurement purpose (*e.g.* research purposes *vs.* process/quality control, measurement time). In general, sizing techniques can be classified into imaging and non-imaging techniques. In imaging techniques some kind of visualization of the particles are reached, and, besides size and size deviation, additional information like shape, morphology, degree of aggregation, that are not available from non-imaging techniques, are also available [56]. Most utilized techniques are light scattering [6] and electron microscope based techniques [57]. Especially for research purposes, and for new samples, combination of two or more techniques, for example light scattering and electron microscopy, are preferred.

3.1.1. Non-imaging techniques

Light scattering techniques are fast, precise, sensitive, and utilizable for a wide particle size area from smallest nanoparticles to micron sized particles. They can be divided into two methods: the Static Light Scattering (SLS) and the Dynamic Light Scattering (DLS) [60]. In SLS, material is illuminated with a light beam and the averaged scattered light intensity is measured over a given time interval at various scattering angles, and based on a scattering theory (model) the information is derived to particle size information. In DLS the fluctuations in the intensity of the light scattered by the particles is measured as a function of time at a fixed angle in order to calculate the particles diffusion coefficient, which in turn can be used to determine their hydrodynamic radius. DLS method is more suitable for submicron particles. The hydrodynamic radius can be affected by the liquid environment during the measurement, especially with small charged particles (< 300 nm), where the electric double layer leads larger particle size results [60]. For example, when mean particle sizes of gold nanoparticles were measured, the mean particle size by transmission electron microscope, TEM, was approximately 50 nm, while the hydrodynamic size in DLS was 60–70 nm [33]. For TEM analysis samples were dried at room temperature, while DLS measurements were done in aqueous solution, and the hydration layer around the nanoparticles in aqueous environment made DLS results larger than TEM values.

Measurements of samples with large particle size deviation, *i.e.* heterogeneous/polydisperse samples, is challenging by light scattering techniques, and in these cases larger particles are overstressed in the

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results. In some cases prehandling is possible, but, if sample prehandling is used, care must be taken in order to properly interpret the results. Especially, if quantified results are needed. Care should be taken that no artefacts are measured: for example in the presence of surfactants (used as stabilizers for drug nanocrystals) micelle formation is possible, which are also measured by DLS.

In light scattering techniques, scattering intensity is dependent on the scattering angle, absorption, particle size, and refractive indices (particle material and dispersion medium) [60]. Uncertainty in any of these parameters can cause alterations in the results. For example, small difference between the refractive indices may cause unreliable results. Also, dynamic processes like Brownian motion or particle aggregation can affect the results. Normally, before the measurements, sample needs to be diluted, and this dilution step may induce some changes in particle size deviation. When extremely fast dissolving drug nanocrystals are diluted, dissolution may take place before measurement and the particle size value is changed towards larger values. In dilution step, saturated drug solution is recommended to be used in order to minimize particle dissolution during dilution step (Fig. 4) [48,62]. Sampling and sample handling are in utmost importance in all the particle size determinations: the more heterogeneous the sample, the more important is reliable sampling. Most of the problems in particle size measurements are due to incorrect sampling and/or sample preparation before the measurement, and extreme care must be followed in order to reach representative samples [63].

In light scattering techniques the particle size information is revealed from the scattering data with the aid of special algorithms, where mostly spherical particle shape approximation is used as a bases [27,62]. When drug nanocrystals are made by top-down techniques, result can be for example needle shaped particles with very different particle dimensions depending on the detection direction. Accordingly, non-spherical particles may lead misleading particle size information: the less the particle size remains spherical, the greater the difference between the measured particle size and actual particle size. This needs to be taken into account, especially, if particle size measurements of differently shaped particles are compared. More precisely, particle shapes, where one dimension differs a lot from the others, like needles, discs or flakes, can be problematic. Particle size analyses are relative measurements. Comparison of values measured by different techniques or even same technique in different laboratories and conditions should be carefully considered.

In DLS, size distribution can be an intensity or volume based distribution [6]. In intensity based measurements, light scattered by each particle is related to the particle size. In volume based measurements contribution of particles is related to their volume. Volume based measurements overstress the fraction of larger particles, and intensity based measurements are recommended for nanoparticulate systems. But, the overstress of larger particles in the volume based measurements can be utilized in comparison of different nanosuspensions, due to its higher sensitivity to the larger particles [6]. In nanoparticle size range, light intensity is proportional to the sixth power of particle diameter, and thus the existence of a small number of larger particles can dominate the particle size results.

3.1.2. Imaging techniques

Electron microscopy techniques, like scanning electron microscopy, SEM, and transmission electron microscopy, TEM, can be utilized for particle sizing, and they give at the same time also particle shape and morphology information [15,64]. SEM requires high vacuum, and clean, dry and electrically conductive samples. This often means that samples needs to be for example platinum or gold coated before the measurement, which can alter the surface characteristics. If sample needs to be dried before analysis, drying method can alter the sample properties, too (Fig. 5). More advanced technique is environmental scanning electron microscopy, ESEM, which does not require high vacuum, and wet or oily samples can be analyzed. However, in most cases with drug nanocrystals and nano-cocrystals, SEM/TEM are recommended techniques for particle shape analysis.

Atomic force microscopy, AFM, can also be utilized for size, shape and morphological studies, though it has not been used as often as electron microscopy, EM, techniques [65]. For purely size determinations statistically relevant number of particles should be measured, and AFM as well as EM techniques are very laborious and time consuming. Techniques are also analyzing only part of the sample. In small particle studies, AFM is more demanding and, hence, less utilized in these purposes. In AFM, a cantilever with a very sharp tip scans the sample surface. When the tip approaches the analyzed surface, the close-range attractive force between the surface and the tip get the cantilever to deflect towards the surface. When the cantilever is further closing the surface (the tip in contact with the surface), increasing repulsive force is acting and the cantilever is deflected away from the surface. Movements of the cantilever are detected by changes in the direction of reflected laser beam, which can be recorded by a position-sensitive photo diode. Topographic map of the sample surface can be formed based on the movements of the cantilever over the scanned surface.

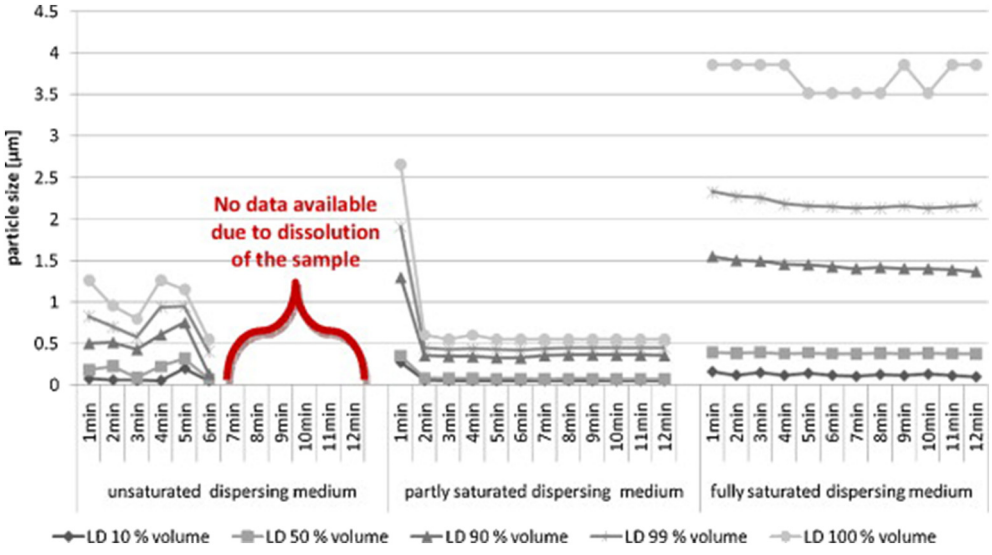

Fig. 4. Particle size measurements for rutin nanosuspensions measured by dynamic light scattering technique in unsaturated (left), partly saturated (middle) and fully saturated (right) dispersion media. Reprinted from [62] with permission from Elsevier.

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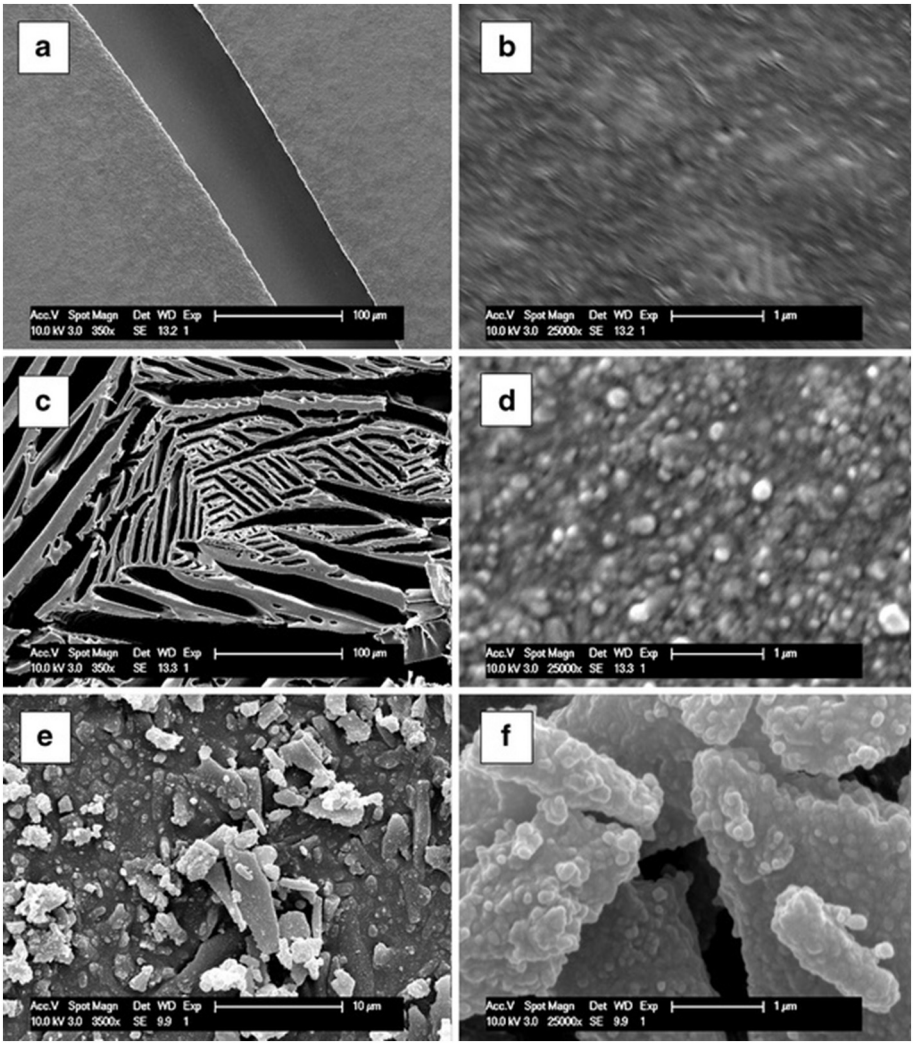

Fig. 5. Scanning electron microscopy figures of loviride nanocrystals with different sample preparations. (a) and (b) drying in air; (c) and (d) freeze-drying; and (e) and (f) dispersion of freeze-dried sample. Reprinted by permission from Springer Nature from [61].

In particle sizing, imaging techniques are too laborous and time consuming for exact particle size analysis. Light scattering based techniques are fast and they should be the first choice for particle sizing. The value of imaging techniques is that they give also the particle shape information, and traditional SEM/TEM are recommended for that analysis. For quality control purposes light scattering measurements alone are enough, but for research purposes and especially with unknown samples combination of imaging technique, like SEM, with light scattering technique, like DLS, is recommended.

3.2. Solid state properties and chemical stability

Solid state form of the material (crystal form, degree or crystallinity, hydrates, solvates, amorphous form) affects physical performance of the system like solubility and dissolution rate. Thermodynamically most stable form has the lowest solubility. Accordingly, exact knowledge of material properties is required [66,67]. Always, when energy input, like temperature changes or mechanical energy, is involved, polymorphic changes or formation of amorphous material is possible, but also chemical degradation may take place.

Solid state analysis and interaction studies can be performed by thermal analysis, mostly differential scanning calorimetry, DSC, or different spectroscopic techniques, like X-ray diffraction, XRD, infrared, IR, and Raman spectroscopy, and less utilized solid state nuclear magnetic spectroscopy, SSNMR. Most standard way to detect chemical purity of the sample is high performance liquid chromatography, HPLC, but liquid chromatography - mass spectrometry, LC-MS, techniques can also be utilized.

When drug nanocrystals are produced *via* top-down techniques, most common techniques being high pressure homogenization and wet milling, the process is typically performed in aqueous dispersion. In these cases, water stabilizes the crystallinity of the drug by acting as a plasticizer [68]. In bottom-up techniques, formation of amorphous material is more typical, especially in liquid atomization based techniques [69]. It is important to be aware of, that, if thermodynamically less stable form is present, changes in solid state form during storage or even during administration are possible, which can alter the biopharmaceutical properties of the system [54,70,71]. In drug nanocrystal studies, DSC and XRD are mostly used techniques for solid state characterization, but also techniques like Raman have been used. Utilization of IR in drug nanocrystal studies is related to recognition of interactions between the drug and excipient(s) in the system.

For cocrystals, it is important to confirm with suitable analytical techniques that drug and coformer coexist in the cocrystals interacting nonionically, and that drug and coformer are present in the unit cell [14]. Regulatory classification of pharmaceutical cocrystals by United States Food and Drug Administration, US FDA, is similar to drug polymorphs and above mentioned proofs are required for new drug applications, NDAs, and abbreviated new drug applications, ANDAs. Pharmaceutical cocrystal analyses are mostly

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performed with XRD, DSC and IR. Also Raman has been utilized and SSNMR, but less frequently.

3.2.1. Thermal analysis

Thermal analysis detects the material properties as a function of temperature. As stated above, most used are different applications of DSC, where by measuring the heat flow, endothermic and exothermic events, like melting point or glass transition temperature, recrystallization, can be recognized. Modulated temperature DSC, MT-DSC, is more sensitive and has higher separation capacity with overlapping thermal events, because it analyses both quasistatic material properties as well as frequency dependency of thermal events. Thermogravimetric analysis, TGA, measures sample mass during the heating, and it is very convenient for example in detecting the exact structure of solvents or hydrates. Case studies demonstrating utilization of thermal analytical techniques in nanosystems characterization are presented in Section 3.2.3.

3.2.2. Spectroscopic techniques

Most common spectroscopic methods in pharmaceutical analysis are different kind of X-ray (XRD and x-ray powder diffraction, XRPD), infrared and Raman techniques. In X-ray techniques, a beam of incident X-rays are diffracted to certain directions, and from the diffractrogram the atomic and molecular level structure of the material can be calculated. IR and Raman are vibrational spectroscopic techniques, where IR operates in IR region and Raman in visible, near IR or near-ultraviolet (UV) wavelengths. For example, interactions between ketoprofen and different polymers (Eudragit RS and Eudragit E) were confirmed by IR analysis; acidic ketoprofen interacted with the ester groups in polymer [72]. Molecules can absorb certain frequencies, which are characteristic to the transition energy of the vibrating bond or functional group in the molecule. Raman measures inelastic scattering, Raman scattering, and it is able to detect vibrational, rotational and other low-frequency transitions in materials.

Solid-state NMR, SSNMR, is not as common as X-ray or DSC in pharmaceutical cocrystal studies, though it can give detailed structural information about cocrystals and complexes [73–76]. Typical production methods for formulation of pharmaceutical cocrystals do not lead to single crystal growth, which may complicate X-ray diffraction studies. SSNMR are sensitive in recognizing molecular associations and structural features like hydrogen bonding. In SSNMR dipolar correlation experiments between spin pairs like $^{1}H—^{1}H$, $^{1}H—^{13}C$, and $^{19}F—^{13}C$ can be utilized to determine hydrogen bonding, intermolecular contacts, and spin diffusion, which link individual molecules together in a crystal structure.

Reliable solid state characterization of material requires utilization of more than one technique, and mostly thermal analyses are combined with some spectroscopic techniques. In following section, case studies demonstrating efficient combination of different techniques in analyzing solid state of drug nanocrystals and nano-cocrystals are presented.

3.2.3. Solid state identification using combination of different techniques

Walsh et al. [77] made several different kinds of cocrystals with sulfadimidine as a drug substance and 4-aminosalicylic acid as a hydrophilic coformer. Solid state characterization of the systems by XRPD and DSC is presented in the Fig. 6. DSC thermograms showed a single endothermic peak characteristic for cocrystal melting point. Melting point of cocrystals made by solvent evaporation technique was 175.84 °C, while melting point for cocrystals produced by spray drying was 170.08 °C. Besides melting point data, also other thermal information like enthalpy of melting was reached from DSC measurements. Extremely fast drying during spray drying induced crystal lattice imperfections, which reflected on the thermal properties of the system. In all the systems only endothermic melting point of cocrystals was seen. No exothermic events were present. When cocrystal components were spray dried together with other excipients, XRPD analysis showed that cocrystal formation took place also in these systems; same characteristic diffraction peaks were seen in all these cocrystal samples.

In IR analysis interactions, like H-bonding, can be recognized (Fig. 7) [77]. If carboxylic acid group is involved in hydrogen bonding in cocrystals, IR is good technique to separate cocrystals from salts [78]. In cocrystals neutral COOH-group shows a strong C═O stretching peak at approximately 1700 cm^{-1} (in salts this peak is not present) and a weak C—O stretching peak approximately at 1200 cm^{-1}. A carboxylate (-COO-) anion in salts has only a single C—O stretching peak in 1000–1400 cm^{-1}. When sulfadimidine - 4-aminosalicylic acid cocrystals were analyzed by attenuated total reflection Fourier transform IR, ATR-FTIR, at 3441 cm^{-1} asymmetric and at 3339 cm^{-1} symmetric stretching bands of -NH_2 group in 4-aminosalicylic acid were detected [77]. Stretching band of NH group of sulphonamide was seen at 3235 cm^{-1}. Hydrogen bonding in cocrystals was confirmed by one broad band at 3482 cm^{-1} and another broad band with a N—H stretching shoulder (NH_2 amine group in 4-aminosalicylic acid) at 3372 cm^{-1}, which both were shifted to lower values from 3493 cm^{-1} and 3386 cm^{-1}, correspondingly. Besides, sulfone stretching, -SO_2, (in sulfadimidine) at 1315 cm^{-1}, and —OH deformation in 4-aminosalicylic acid at 1275 cm^{-1} confirmed in this case the formation of cocrystals and that the formation was based on hydrogen bonding.

Kumar and Burgess [79] studied naproxen nanocrystals made by wet milling process. In modulated temperature DSC analysis they found that increased amount of stabilizer (hydroxypropyl methyl cellulose, HPMC) was seen in the thermogram as a lower melting temperature of naproxen indicating molecular level interactions between naproxen and cellulose. Also in IR analysis peak shift were seen indicating formation of strong bond or molecular level interactions in the system.

When spironolactone nanocrystals were made with four different stabilizers (poloxamers 188 and 407, HPMC and sodiumdeoxycholate), spironolactone was in crystalline form II based on DSC analysis [80]. In Raman analysis pure spironolactone showed four characteristic peaks in the double bond region: C═O stretching of lactone ring (1766 cm^{-1}), thioacetyl group (1690 cm^{-1}), C_6 ring (1670 cm^{-1}) and C═C stretching (1616 cm^{-1}), while the excipients did not have any peaks in that area. Drug-excipient interaction studies were based on the characteristic drug peaks: if characteristic peaks are not shifted, there are no interactions between the drug and excipient. Peaks at 1690–1670 cm^{-1} disappeared with Poloxamer 407 stabilized spironolactone nanocrystals confirming drug stabilizer interactions. With other excipients no peak shift/disappearance and hence no interactions were noticed.

Changes in piroxicam crystal form during nanocrystallization by high pressure homogenization were confirmed by XRD, FTIR and DSC analysis [81]. The piroxicam raw material was in crystal form I and white in color. After the nanocrystallization, nanosuspension was yellow in color and it was found to be a mixture of crystalline monohydrate and form III; monohydrate is yellow powder, which was already seen in the colored appearance of the particles. In this case the solubility difference between the different crystalline forms of BCS class II drug piroxicam was evident: the solubility of form I is 14.3 mg/L as compared to solubility value of 17.0 mg/L for form III.

Carbamazepine is well-known example of a drug existing in many different crystal forms. When carbamazepine nanocrystals were produced by electrospraying technique, XRD and DSC analysis showed that drug was in amorphous form [69]. After fast water absorption from the air, drug was transformed to anhydrated form. Eight hours after production, XRD analysis showed large amorphous halo, but also small peaks characteristic for form III were found. Further, after 12 h characteristic peaks for form I were also seen, and during storage the relative amounts of form I and form III changed again. Accordingly, the storage stability of the system was poor, and transformation towards more thermodynamically stable solid state form of the drug took place fast during storage.

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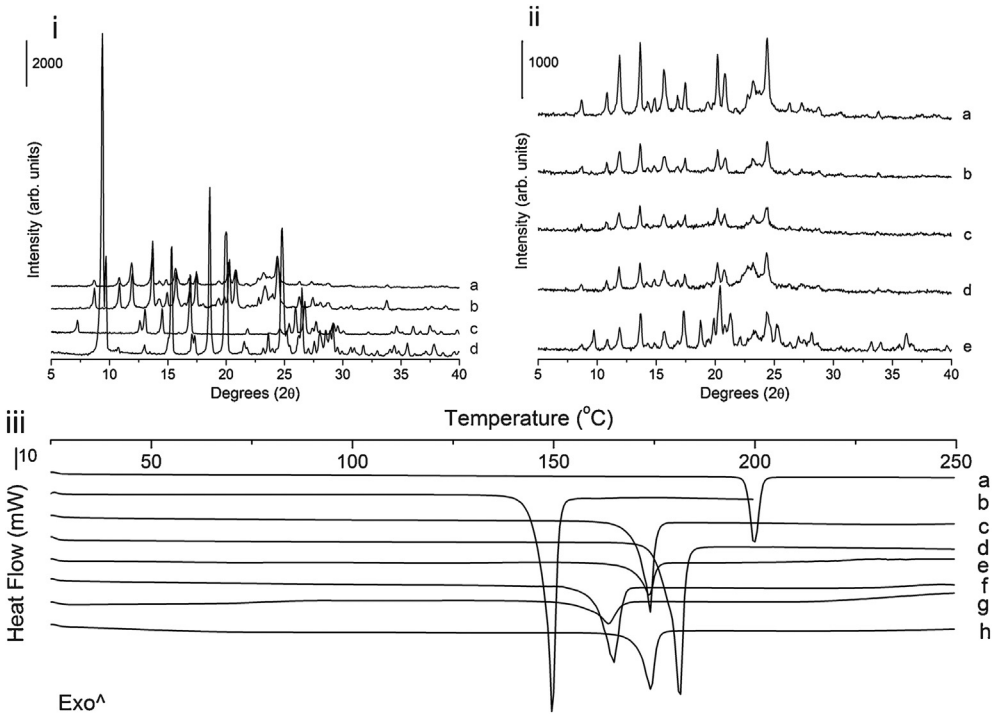

Fig. 6. XRPD diffractograms and DSC thermograms of sulfadimidine cocrystals and co-spray dried systems. (i) XRPD of a) Cocrystals by spray drying, b) Cocrystals by slow solvent evaporation, c) 4-aminosalicylic acid (coformer), d) sulfadimidine (drug). (ii) XRPD of co-spray dried systems with excipient: a) cocrystal by spray drying, b-e) cocrystal components co-spray dried with b) dextran, c) inulin, d) MCC, e) mannitol. (iii) DSC thermograms of a) sulfadimidine, b) 4-aminosalicylic acid, c) cocrystals by spray drying, d) cocrystals by solvent evaporation, e-h) cocrystal components co-spray dried with e) inulin, f) mannitol, g) MCC, h) dextran. Reprinted from [77] with permission from Elsevier.

Also other kind of interactions can take place in drug-excipient systems, and sometimes drug and excipient can form eutectic mixtures, which can be detected by suitable analytical techniques. Formation of eutectic mixture between ibuprofen and poloxamer in nanosuspension was confirmed by DSC analysis [82]. The exothermic melting peak of pure ibuprofen was at 74.8 °C, and for pure poloxamer at 51.4 °C. For the nanosuspension, melting peak for eutectic mixture was seen at 39.4 °C and melting peak for the excess ibuprofen was seen at 56.8 °C.

Sander et al. [15] formulated caffeine and 2,4-dihydroxybenzoic acid monohydrate nano-cocrystals by sonication process. In this case drug and coformer made two-dimensional graphite like structure based on intramolecular (OH (hydroxy group)—O (carboxy group)) and intermolecular (OH (carboxy group)—N (imidazole group)) hydrogen bonding. Particle size and size deviation was determined by DLS, and SEM confirmed the particle shape and morphology. Calculated diffractogram for the cocrystals gave peaks (2θ) at 10.78°, 12.98°, 17.48°, 21.58°, and 22.98°, and XRPD analysis confirmed successful formation of cocrystals. SEM analysis showed formation of plate shaped nanoparticles, and DLS measurements showed with the best formulation average particle size to be 136 nm with polydispersity index (PDI) value of 0.239, meaning formation of small and monodisperse drug nano-cocrystals.

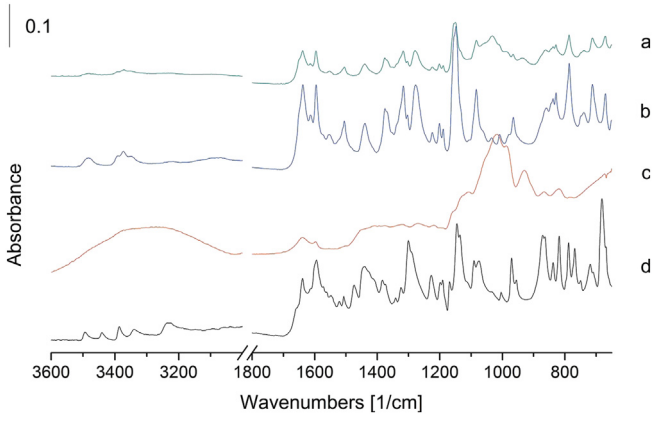

Fig. 7. FTIR analyses of (a) co-spray dried sulfadimidine −4-aminosalicylic acid cocrystals in inulin, (b) spray dried cocrystals, (c) inulin, (d) a physical mixture of sulfadimidine and 4-aminosalicylic acid. Reprinted from [77] with permission from Elsevier.

Spitzer et al. [65] made caffeine-oxalic acid (2:1 M ratio) and caffeine-glutaric acid (1:1 M ratio) nano-cocrystals by spray-flash evaporation process, where the crystallization rate is extremely fast. Particle size, measured by atomic force microscope, AFM, was for caffeine-glutaric acid cocrystals 111 nm and shape was acicular. Formation of cocrystals was confirmed by XRD: diffractograms differed those of the individual components and corresponded well with the theoretical patterns from Cambridge Structural Database and literature references. From XRD results the coherent crystallite sizes were calculated from the full width at half maximum (FWHM) of the X-ray signals. For both the systems the formed nanoparticles contained five to ten single crystals, because particle size values determined by AFM were approximately 2–3 times bigger than the corresponding coherent crystallite size values. In the same study cocrystalline explosives, 2,4,6-trinitrotoluene - 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (TNT-HMX), were tried to formulate; in XRD analysis of the system the active agent peaks were present but the coformer peaks were lacking. This system was semi-crystalline in nature, not cocrystals: raw materials were only physically mixed, not chemically combined *via* molecular level interactions, like is the case with cocrystals.

Further analyses were performed and DSC analysis confirmed the above mentioned XRD results [65]. In TNT-HMX sample, which instead of cocrystals formed physical mixture, the TNT melting peak was seen in DSC thermogram. In DSC, heating and cooling cycles were repeated. When, after the first DSC heating, the system was cooled and heated again, the TNT melting peak was similar to the peak in the first heating. For another cocrystal system, carreine-oxalic acid nano-cocrystals, first heating gave single melting peak for cocrystal, which was between the

melting temperatures of the raw materials. After the melting, pure oxalic acid was decomposed, when it was released from the cocrystal structure. During the second heating, the melting peak of pure caffeine was seen. Raman spectra further confirmed the DSC and XRD results, when the Raman bands fitted to the published cocrystal vibrational bands.

Vogt et al. [73] made SSNMR analysis for a number of well-known pharmaceutical cocrystals, which were already characterized by XRD. Formation of all the studied cocrystals was based mainly on hydrogen bonding and ^{1}H SSNMR was a powerful tool, because it was able to selectively detect hydrogen bonds. For example, formation of binary cocrystals of palmitic acid and nicotinamide was based on hydrogen bonding between carboxylic acid donor of palmitic acid and pyridine acceptor in nicotinamide, which was detected by SSNMR. This structure is common in cocrystals based on carboxylic acids and nitrogen bases. SSNMR studies utilized a combination of 1D and 2D dipolar correlation methods. Especially, in complex systems the SSNMR showed better sensitivity as compared to XRD, where some of the peaks were overlapping.

In another study theophylline cocrystal structures were confirmed by combining SSNMR and FTIR [74], when hydrogen bond formation between theophylline and cocrystal former (carboxylic acid) was found in SSNMR spectra. Two polymorphic forms of ethenzamide-gentisic acid cocrystals were characterized by ^{13}C and ^{15}N SSNMR and FTIR [75] and again changes in ^{13}C and ^{15}N chemical shift values for ethenzamide molecule were observed, which confirmed intramolecular hydrogen bonding in cocrystals. Formation mechanisms of caffeine-malonic acid cocrystals were tracked in real time by ^{13}C SSNMR [76].

In interpretation of nanoparticle analysis, it should be kept in mind that with smallest nanosized particles melting temperature (for example in DSC analyses) can be slightly shifted to lower temperatures, because the free energy of the system is higher and crystal energy lower [48,80]. For example with valsartan, when the long-range order of crystals was tens of nanometers, the lowering of melting temperature by >20 °C was noticed as compared to normal sized valsartan crystals [83]. In the same way with smallest particle size fractions peak broadening or lowered intensities can be seen in X-ray diffractograms [6,63]. This was demonstrated in a study by Aleandri et al. [63], where, based on the DSC studies, fenofibrate and bezofibrate nanocrystals were in crystalline form, but the diffraction intensities in X-ray diffractograms were slightly decreased. The peak intensities were lowered due to the decreased particle size in nanoscale and/or disorders in crystal structure, which were more pronounced in nanosized particles.

In solid state characterization, XRD is the golden standard. It has been utilized for a long time and a lot of reference difractograms are available in the literature. In combination with DSC, it gives enough data for reliable solid state characterization, and that combination is recommend for solid state analysis. Benefits of DSC are small sample amount, but the controlled heating during the analysis run may induce changes in the sample. Raman is not as universal for all kind of the molecules as XRD, and it also is less frequently used. If the laser power in spectroscopic techniques is high or the measurement time long, sample can be burned, which can affect the results. In interaction studies IR is powerful tool and first choice; if more precise information related to hydrogen bonding is needed, SSNMR is good option, though it cannot be recommended as a standard analysis technique.

3.2.4. Chemical stability

All the above-described techniques can give some information also about the chemical purity or degradation of the drug, but most standard way to detect this is high performance liquid chromatography, HPLC. Impurities or degradation products are seen in HPLC as new peaks, but recognition of side products often requires other techniques like liquid chromatography - mass spectrometry, LC-MS. Degradation can be induced by harsh process conditions, and exact knowledge of chemistry of the unwanted products is crucial in order to recognize the problematic process steps.

Degradation of naproxen during nanomilling was studied with two stabilizers; hydroxypropyl methyl cellulose, HPMC, and Tween 80 [79]. Tween 80 stabilized nanocrystals were stable, but milling with HPMC induced molecular level interactions between drug and stabilizer, which were seen in MT-DSC analysis. Chemical degradation was confirmed in HPLC analysis (Fig. 8), when extra degradation peaks were present. In order to recognize the degradation product, LC-MS analyses was run, and the degradation product peak was seen at 208 *m/z*. Peak intensity, *i.e.* degradation product concentration, was increased with longer milling times. Based on literature and earlier studies, the most common degradation product of naproxen was identified to be decarboxylated naproxen [84], and the LC-MS peak corresponded sodium salt of decarboxylated naproxen.

Chemical stability and drug content analysis of itraconazole nanocrystals prepared by sonoprecipitation technique were confirmed by HPLC analysis [85]. After 3 months storage, the drug content had not changed significantly, and no degradation peaks were noticed in HPLC graph. When paclitaxel nanocrystals were produced by laser irradiation technique, particle size was approximately 400 nm with narrow particle size deviation [86]. However, the process was associated with significant chemical degradation of paclitaxel due to high laser power. The amount of paclitaxel and its degradation products in nanosuspension after laser treatments was evaluated by HPLC technique and 23.1% of paclitaxel was degraded.

Chemical stability of adefovir dipivoxil was improved by formation of cocrystals with saccharin [87]. After 18 days of storage of the pure drug, the total amount of impurities was 90.71%. Adefovir dipivoxil – saccharin cocrystals had a significantly higher drug content and a significantly lower content of impurities at all the sampling points. After 47 days of storage, no significant content change was found in adefovir dipivoxil–saccharin cocrystals; the total amount of impurities was <2%.

3.3. Drug release and solubility

For drug cocrystals, one should demonstrate that the pure drug is released from the cocrystal structure *in vivo*; for that, *in vitro* dissolution and/or solubility testing has been considered to be sufficient [14]. Also, reliable dissolution method is crucial performance testing for drug nanocrystals in order to confirm expected solubility and drug release profiles. Both drug nanocrystals as well as nano-cocrystals are considerably simple in structure, and their ability to improve the solubility properties are well known *in vitro*. However, fast dissolution *in vitro* does not necessarily lead to enhanced permeation *in vivo*, for example due to drug precipitation before the adsorption, and this poses extra requirement for finding a reliable and correlative drug release testing platform for these systems. For drug nanosystems no compendial standards for drug release testing exist, and currently different methods have been utilized, both including (modified) pharmacopoeial methods as well as new techniques [88].

3.3.1. Apparent solubility and supersaturation

In the same way than with the other high energy state systems, like amorphous systems or metastable polymorphic forms, with both the drug nanocrystals and nano-cocrystals, the apparent solubility levels reached in the dissolution testing are higher than the thermodynamic solubility of the bulk drug. For example, apparent solubility in 0.5% aqueous sodium dodecyl sulphate solution for fenofibrate nanocrystals (size 460 nm) was 67.51 g/mL while the corresponding value for bulk drug (particle size 80 μm) was only 6.02 g/mL [89]. Higher apparent solubility values can cause drug precipitation in more thermodynamically stable form, *i.e.* less soluble form, after the initial fast dissolution [47,90]. This needs to be avoided/minimized by careful formulation planning in order to reach the biopharmaceutical benefits with these systems *in vivo*.

Further, this uncontrolled precipitation takes place on top of the nanocrystals/nano-cocrystals, which even lowers the solubility of

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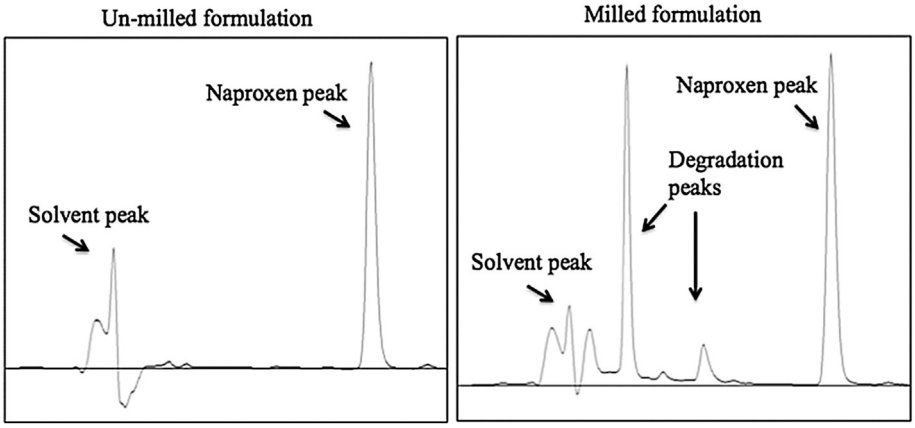

Fig. 8. Degradation peaks of hydroxypropyl methylcellulose, HPMC, stabilized naproxen nanocrystals in HPLC. (Time is presented in x-axis and peak intensity in y-axis.) Reprinted from [79] with permission from Elsevier.

remaining nanosystems, and, leads to the case that the solubility of the system equals to the solubility of the precipitated drug form. One strategy to avoid uncontrolled precipitation of already dissolved nanocrystals and nano-cocrystals is the use of crystallization inhibitors in the formulation, *i.e.* polymers like HPMC or PVP [4,91–94]. In the case of cocrystals, also excess amount of coformer can be used [95,96].

Higher apparent solubility and extremely fast dissolution rate with these systems poses extra requirements for reliable *in vitro* dissolution testing [11,97]. In small particle systems, particle size is highly related to the dissolution rate and apparent solubility [28] and in order to reach discriminating dissolution results, *in vitro* dissolution test can be performed under non-sink conditions. Another reason for non-sink conditions *in vitro* is the evaluation of their ability to maintain supersaturated state (solution stability), and the possibility to predict *in vivo* performance of the system by utilizing testing under these stressed conditions. But, care must be taken in order to interpret the results correctly [11,97,98].

Generally, drug release data for supersaturated systems under non-sink conditions are recognized as an evaluation method for their ability to maintain supersaturated state (solution stability). Supersaturation can also enhance the permeation; it was shown that the higher concentration increased the membrane transport rate both with artificial and biological membranes [99,100], reaching the maximum permeation with the amorphous solubility value, where the liquid-liquid phase separation started [101,102].

Maintenance of supersaturation with different kinds of supersaturating systems, namely carbamazepine nanocrystals and amorphous carbamazepine, was measured in real-time detection by ^{1}H NMR technique [103]. For carbamazepine nanocrystals (size 150 nm) dissolved concentration was almost constant during 50 h time, while for amorphous carbamazine initial concentration was higher, but it was then lowered below the stable concentration value with drug nanocrystals. The lower level of supersaturation reached with drug nanocrystals was more stable, while with amorphous drug the higher level of supersaturation was kinetically less stable leading to precipitation. Shiraki et al. [104] formulated exemestane-maleic acid and megestrol acetate-saccharin cocrystals. While both improved intrinsic dissolution rate as compared to the original bulk drug materials, exemestane cocrystals were transformed to pure drug after 1 min time in suspension, while transformation for megestrol acetate cocrystals took 2–4 h in suspension, indicating higher stability (Fig. 9). Solid state forms were confirmed by XRPD technique.

Further challenges are faced *in vivo*, when for example pH changes in gastro intestinal, GI, tract may alter the level of supersaturation drastically and induce uncontrolled precipitation [53]. For example, solubility of itraconazole is approximately 250-times higher in stomach (acidic pH) than in intestine (slightly basic pH). *In vitro* the dissolution of itraconazole from drug nanocrystals was superior as compared to commercial Sporanox® granules. However, when itraconazole nanocrystals were packed in capsules and *in vivo* tests were performed with rats, fast dissolution of drug nanocrystals was followed by fast precipitation. After dissolution drug in solution was immediately transited to intestine, where the solubility of itraconazole was much lower, and fast precipitation took place. Fast precipitation inhibited absorption, and the area under the curve, AUC, value with nanocrystals was lower as compared to commercial Sporanox® granules. Hence, *in vitro – in vivo* correlation, IVIVC could not be established. When itraconazole nanocrystals were attached to cellulose matrix, enhanced bioavailability was realized [7]. Accordingly, with supersaturating systems in order to reach reliable IVIVC, the importance of dissolution testing set-up cannot be overemphasized.

In order to evaluate the apparent solubility values and concentration level differences between different nanoparticle size fractions, Sarnes et al. [105] utilized UV imaging and channel flow methods for solubility studies of indomethacin nanocrystals. In both techniques, nanocrystals were compressed into tablets and in the both set-ups, flat tablet surfaces were flushed with medium. Accordingly, particle size effect on solubility was neglected, and intrinsic dissolution values were measured. In UV-imaging, concentration profiles on the compression surfaces showed surface concentration value for drug nanocrystals (size 580 nm) being 28.7 mg/L, while the corresponding value for bulk drug surface was as low as 2.1 mg/L. Drug concentration profiles as a function of distance from the compact surface for different particle sizes and at different time points are shown in Fig. 10. In channel flow equipment intrinsic dissolution rates were clearly different even between different nanosized particle fractions, too.

3.3.2. Dissolution testing

The official pharmacopoeia dissolution methods (European, Japanese, United States Pharmacopoeia) are best for quality control purposes and they are material and time consuming [106,107]. Often also with fast dissolving samples, their sensitivity is not high enough. Instead, different imaging-based techniques, like UV, FTIR, near infrared (NIR), magnetic resonance imaging (MRI), Raman and coherent anti-Stokes Raman scattering (CARS) methods, which can give more precise information about the dissolution process can be utilized or they can be combined to traditional pharmacopoeia techniques [70,105,108–113]. Different kind of approach for dissolution studies was presented by Kayaert et al. [114], when they measured dissolution rate of three different drugs by solution calorimetry based on the temperature changes in the reaction vessel. Temperature data were transformed to heat of solution. All the drugs dissolved totally in <1 min time. Calorimetry measures total heat production and consumption, and this can lead misleading interpretations. For example, separating the heat of mixing from the heat of solution can be impossible.

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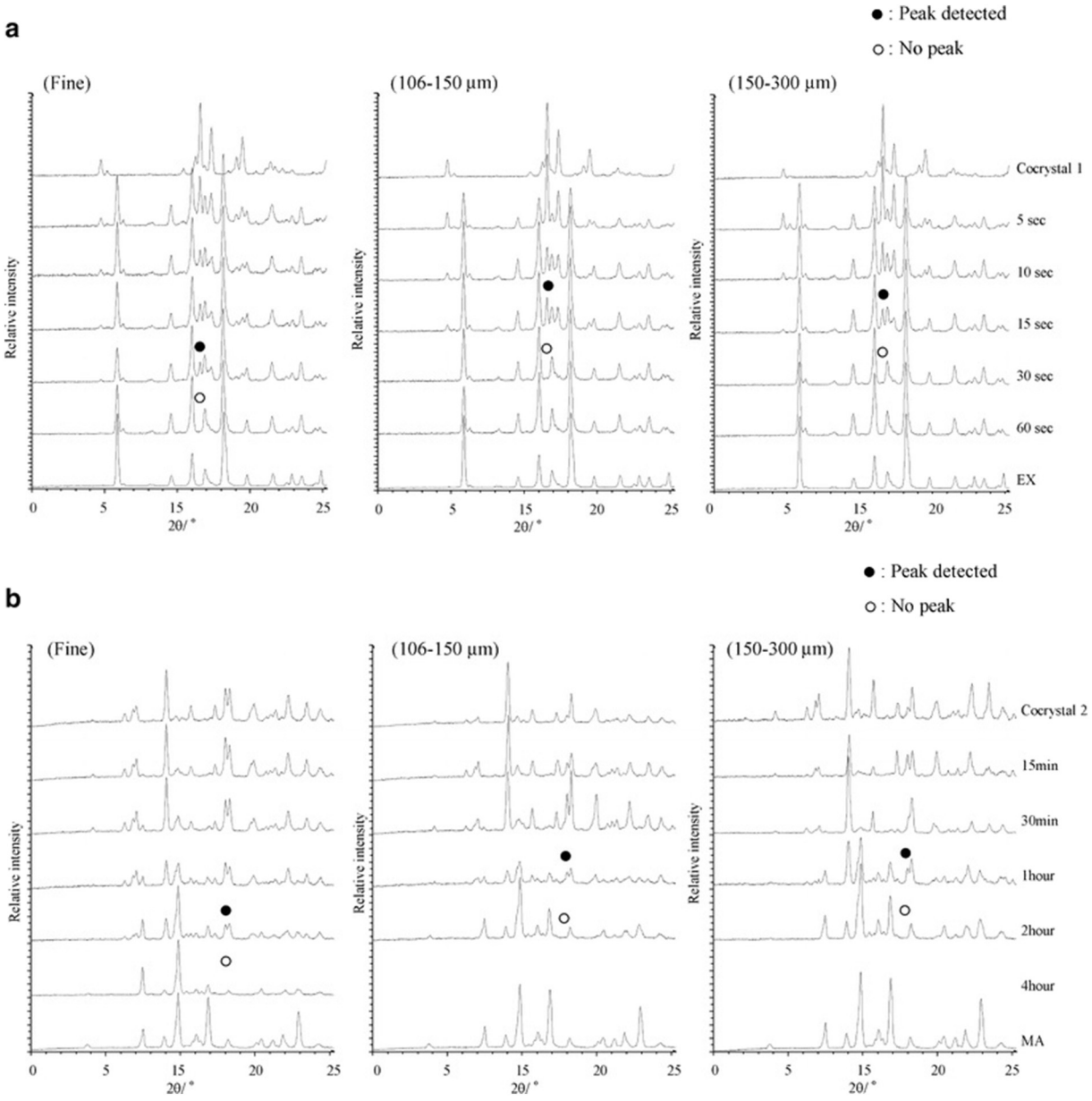

Fig. 9. XRPD diffractograms of (a) exemestane-maleic acid, and (b) megestrol acetate-saccharin cocrystals in suspension with different particle size fractions. XRPD confirms transformation from cocrystals to pure drug. Reprinted by permission from Springer Nature from [104].

Official pharmacopoeia methods can be usable for nanoparticle dissolution testing, if the test conditions are tailored. Liu et al. [97] modelled dissolution mechanisms for polydisperse nanoparticles in order to find the best discriminating dissolution environment. Simulations showed that when the sample amount equaled to the saturation solubility of the measured sample, the discrimination power was best between different nanosized fractions. When the concentration was further increased, the discrimination power was lowered. Simulation results were confirmed experimentally in pharmacopoeia basket method. Accordingly, selection of dissolution medium and amount of medium/sample are important factors.

Often the challenge in nanoparticle dissolution and solubility testing is the separation of colloidal sized particles from the liquid phase before the concentration measurements. Mostly utilized techniques for separation are ultracentrifugation [48] and filtration [115] or both together [8]. In filtering the smallest particles may pass through the filter or particles can stack on the filter. Often in filtering only the pore size is taken into account. But, also interactions between the drug and filter material should be taken care of. In centrifugation small nanoparticle require high forces and long process times and dissolution may continue even during the centrifugation or drug can be absorbed to the tube material.

When filtration with two different filter types and ultracentrifugation were compared as separation techniques for dissolution studies of indomethacin nanocrystals, it was noticed that indomethacin was attached to both the filter materials as well as to the centrifugation tubes [97]. In that study, the filter still was the most reliable technique, when the filter type was carefully selected. If drug is interacting with filter material, it is typical that during the filtering the first drops of the sample has lower concentration, because the drug is attaching to the filter material. When the filter material is saturated with the drug, the concentration of the filtrate is higher. In order to check the presence of solid particles in the liquid sample, UV-spectrophotometric analysis with two different wavelengths can be beneficial [105]: one wavelength at the drug absorption maximum for drug concentration determinations and the other wavelength were the absorbance of drug and excipients is negligible for confirmation of absence of undissolved particles.

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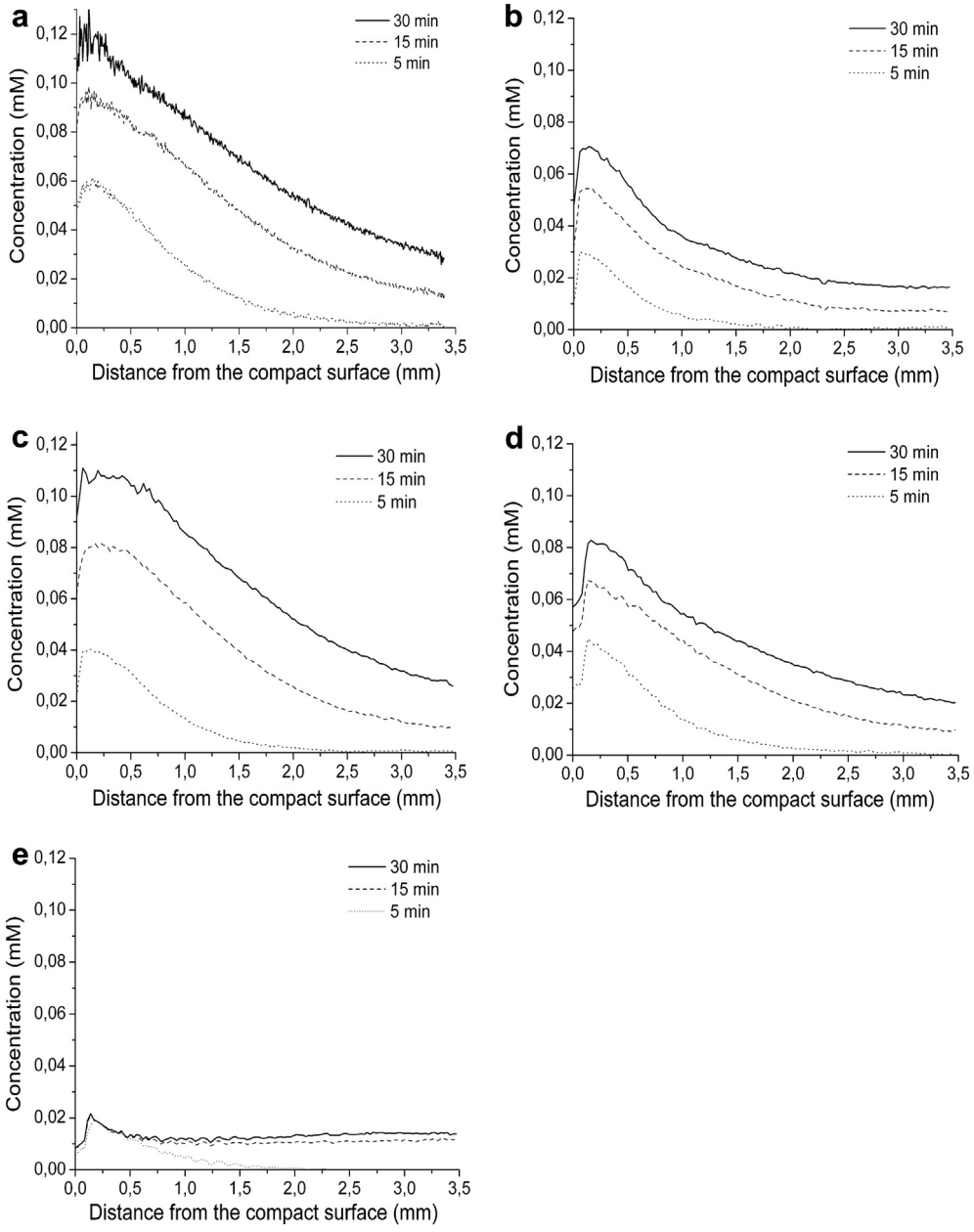

Fig. 10. Apparent drug concentration-distance from the compact surface -profiles by UV imaging for indomethacin nanoparticles with two different stabilizers (poloxamers F68 and F127) at time points of 5, 15 and 30 min. (A) F68/particle size 580 ± 30 nm, (B) F68/micronsized particles, (C) F127/particle size 580 ± 20 nm, (D) F127/micronsized particles and (E) bulk drug/particle size tens of μm. Reprinted from [105] with permission from Elsevier.

In nanoparticle studies, dialysis bags or other kind of dialysis methods can be used in dissolution tests. For example, drug is put inside of a dialysis bag, which is analyzed in pharmacopoeia basket or paddle setups. However, if dialysis bags/membranes are used, care must be taken in the membrane selection in order to avoid that the limited membrane transport is in fact controlling the drug release [116,117]. This is often the problem with fast dissolving systems, like drug nanocrystals and nano-cocrystals, or if there is a burst release in the beginning of the dissolution process [118]. Drug can also interact with the membrane material in the same way than with filters.

Drug release testing is important but demanding part of analysis for nanosized drug systems, and no compendial or standard universal procedure exist for them [84,119]. Different techniques are available, pharmacopoeia methods can be used, but often measurement conditions need to be tailored case by case based on the aims of the testing (for example, data for purely research purposes or quality control purposes). Supersaturated drug delivery systems, like drug nanocrystals and nano-cocrystals are extra demanding and may cause problems in IVIVC. If the tests are performed under sink conditions *in vitro*, IVIVC can be difficult to establish [53].

4. Conclusions

Physical stability (stable particle size and solid state form), solubility behavior, and drug release testing are the most important functional properties of drug nanocrystals and nano-cocrystals.

Particle size analysis can be performed by imaging or non-imaging techniques. Non-imaging techniques are fast, reliable, and well suitable for process control purposes. Sample heterogeneity may cause misleading results, and it is advisable to confirm the particle size and shape information with some imaging technique, most preferable by electron microscope technique.

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Solid state characterization can be done by spectroscopic or thermic analysis. The more complicated the system is, for example the higher number of excipients, more probable it is that part of the information is overlapping each other. In order to reach reliable information of solid state form, it is recommended to use more than one technique, recommended combination is DSC as a thermal analysis and x-ray analysis. For drug nanocrystals, solid state confirmation is enough, but for nano-cocrystals, requirement is confirmation of formation of cocrystal structure. Standard method for interaction studies confirming the cocrystal structure is IR techniques, but so far less utilized SSNMR is also very good technique especially in recognizing hydrogen bonding.

In solubility and dissolution studies the aim of the studies should be recognized. From these nanosystems dissolution is often so fast that compendial methods do not have enough discriminative power for detecting differences between the samples. Also supersaturating drug delivery systems, like drug nanocrystals and nano-cocrystals pose special requirements for *in vitro* test methods if IVIVC correlation needs to be found.

In the first screening studies with new chemical entity the sample amount is very small. This states extra demands for the reliable analysis, when only a limited number of tests can be done. On the other hand, fast and reliable information, for which the decision is based on, are highly required. The importance of reliable and thorough *in vitro* characterization of nanosystems can never be overemphasized. In order to reach good IVIVC, mimicking of *in vivo* conditions is crucial. In nanosystems even very small changes can have dramatic consequences *in vivo* bioavailability, which may not be visible *in vitro* tests. Small scale sets extra demands to the analytics and for example for drug release testing no compendial or standard test protocol exist, which further increases analytical challenge.

New techniques open up new possibilities, like for example new spectroscopic techniques utilized in solid state analysis. These techniques can give more exact information for research purposes, but in industrial quality control purposes they are not the first choice. They require much more research evidence to be suitable for every day process control purposes. Traditional and well established techniques are recommended for quality control purposes.

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References

[1] C.M. Keck, R.H. Müller, Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization, Eur. J. Pharm. Biopharm. 62 (2006) 3–16.
[2] R.H. Müller, S. Gohla, C.M. Keck, State of the art of nanocrystals – special features, production nanotoxicology aspects and intracellular delivery, Eur. J. Pharm. Biopharm. 78 (2011) 1–9.
[3] J. Möschwitzer, Drug nanocrystals in the commercial pharmaceutical development process, Int. J. Pharm. 453 (2013) 142–156.
[4] L. Peltonen, J. Hirvonen, Drug nanocrystals – versatile option for formulation of poorly soluble materials, Int. J. Pharm. 537 (2018) 73–83.
[5] X. Yang, Y. Liu, Y. Zhao, M. Han, Y. Guo, H. Kuang, X. Wang, A stabilizer-free and organic solvent-free method to prepare 10-hydrozycamptothecin nanocrystals: in vitro and in vivo evaluation, Int. J. Nanomedicine 11 (2016) 2979–2994.
[6] A.M. Cerdeira, M. Mazzotti, B. Gander, Formulation and drying of miconazole and itraconazole nanosuspensions, Int. J. Pharm. 443 (2013) 209–220.
[7] H. Valo, M. Kovalainen, P. Laaksonen, M. Häkkinen, S. Auriola, L. Peltonen, M. Linder, K. Järvinen, J. Hirvonen, T. Laaksonen, Immobilization of protein-coated drug nanoparticles in nanofibrillar cellulose matrices – enhanced stability and release, J. Control. Release 156 (2011) 390–397.
[8] H. Valo, S. Arola, P. Laaksonen, M. Torkkeli, L. Peltonen, M.B. Linder, R. Serimaa, S. Kuga, J. Hirvonen, T. Laaksonen, Drug release from nanoparticles embedded in four different nanofibrillar cellulose aerogels, Eur. J. Pharm. Sci. 50 (2013) 69–77.
[9] V.K. Pawar, Y. Singh, J.G. Meher, S. Gupta, M.K. Chourasia, Engineered nanocrystal technology: in-vivo fate, targeting and applications in drug delivery, J. Control. Release 183 (2014) 51–66.
[10] N. Qiao, M. Li, W. Schlindwein, N. Malek, A. Davies, G. Trappitt, Pharmaceutical cocrystals: an overview, Int. J. Pharm. 419 (2011) 1–11.
[11] G. Kuminek, F. Cao, A.B. de Oliveira da Rocha, S.G. Cardoso, N. Rodriguez-Hornedo, Cocrystals to facilitate delivery of poorly soluble compounds beyond-rule-of-5, Adv. Drug Deliv. Rev. 101 (2016) 143–166.
[12] D.J. Berry, J.W. Steed, Pharmaceutical cocrystals, salts and multicomponent systems; intermolecular interactions and property based design, Adv. Drug Deliv. Rev. 117 (2017) 3–24.
[13] A.M. Healy, Z.A. Worku, D. Kumar, A.M. Madi, Pharmaceutical solvates, hydrates and amorphous forms: a special emphasis on cocrystals, Adv. Drug Deliv. Rev. 117 (2017) 25–46.
[14] FDA, Regulatory classification of pharmaceutical co-crystals guidance for industry, 2018https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM281764.pdf.
[15] J.R.G. Sander, D.-K. Bucar, R.F. Henry, G.G.Z. Zhang, L.R. MacGillivray, Pharmaceutical nano-cocrystals: sonochemical synthesis by solvent selection and use of a surfactant, Angew. Chem. Int. Ed. 49 (2010) 7284–7288.
[16] R. Thakuria, A. Delori, W. Jones, M.P. Lipert, L. Roy, N. Rodriguez-Hornedo, Pharmaceutical cocrystals and poorly soluble drugs, Int. J. Pharm. 453 (2013) 101–125.
[17] J. Lu, S. Rohani, Preparation and characterization of theophylline–nicotinamide cocrystal, Org. Process. Res. Dev. 13 (2009) 1269–1275.
[18] N. Schultheiss, A. Newman, Pharmaceutical cocrystals and their physicochemical properties, Cryst. Growth Des. 9 (2009) 2950–2967.
[19] J.F. Remenar, S.L. Morissette, M.L. Peterson, B. Moulton, J.M. MacPhee, H.R. Guzmán, Ö. Almarsson, Crystal engineering of novel cocrystals of a triazole drug with 1,4-dicarboxylic acids, J. Am. Chem. Soc. 125 (2003) 8456–8457.
[20] M. Li, N. Ioannidis, C. Gogos, E. Bilgili, A comparative assessment of nanocomposites vs. amorphous solid dispersions prepared via nanoextrusion for drug dissolution enhancement, Eur. J. Pharm. Biopharm. 119 (2017) 68–80.
[21] A. Avdeef, Cocrystal solubility product analysis – dual concentration-pH mass action model not dependent on explicit solubility equations, Eur. J. Pharm. Sci. 110 (2017) 2–18.
[22] A. Avdeef, Cocrystal solubility product prediction using an in combo model and simulations to improve design of experiments, Pharm. Res. (2018)https://doi.org/10.1007/sl1095-018-2343-3.
[23] A. Dalpiaz, V. Ferretti, V. Bertolasi, B. Pavan, A. Monari, M. Pastore, From physical mixtures to co-crystals: how the coformers can modify solubility and biological activity of carbamazepine, Mol. Pharm. 15 (2018) 268–278.
[24] T. Rager, R. Hilfiker, Cocrystal formation from solvent mixtures, Cryst. Growth Des. 10 (2010) 3237–3241.
[25] E. Gagniere, D. Mangin, F. Puel, J.-P. Valour, J.-P. Klein, O. Monnier, Cocrystal formation in solution: inducing phase transition by manipulating the amount of cocrystallizing agent, J. Cryst. Growth 316 (2011) 118–125.
[26] K. Sigfridsson, P. Skantze, U. Skantze, L. Svensson, L. Löfgren, P. Nordell, E. Michaëlsson, B. Smedsrød, B. Fuglesteg, K. Elvevold, Nanocrystal formulations of a poorly soluble drug. 2. Evaluation of nanocrystal liver uptake and distribution after intravenous administration to mice, Int. J. Pharm. 524 (2017) 248–256.
[27] L. Peltonen, J. Hirvonen, Physicochemical characterization of nano- and microparticles, Curr. Nanosci. 4 (2008) 38–43.
[28] L. Peltonen, J. Hirvonen, J.T. Laaksonen, Drug nanocrystals and nanosuspension in medicine, in: V. Torchilin (Ed.), Handbook of Nanobiomedical Research, Fundamentals, Applications and Recent Developments, 1 Materials for Nanomedicine, 3, World Scientific, Singapore 2014, pp. 169–197.
[29] M.A. Raza, Z. Kanwal, A. Rauf, A.N. Sabri, S. Riaz, S. Naseem, Size- and shape-dependent antibacterial studies of silver nanoparticles synthesized by wet chemical routes, Nano 6 (2016) 74.
[30] A.B. Jindal, The effect of particle shape on cellular interaction and drug delivery applications of micro- and nanoparticles, Int. J. Pharm. 532 (2017) 450–465.
[31] Arnida, M.M. Janát-Amsbury, A. Ray, C.M. Peterson, H. Ghandehari, Geometry and surface characteristics of gold nanoparticles influence their biodistribution and uptake by macrophages, Eur. J. Pharm. Biopharm. 77 (2011) 417–423.
[32] X. Xie, J. Liao, X. Shao, Q. Li, Y. Lin, The effect of shape on cellular uptake of gold nanoparticles in the forms of stars, rods, and triangles, Sci. Rep. 7 (2017) (2017), 3827.
[33] N. Darville, J. Saarinen, A. Isomäki, L. Khriachtchev, D. Cleeren, P. Sterkens, M. van Heerden, P. Annaert, L. Peltonen, H.A. Santos, C.J. Strachan, G. Van den Mooter, Multimodal non-linear optical imaging for the investigation of drug nano−/microcrystal-cell interactions, Eur. J. Pharm. Biopharm. 96 (2015) 338–348.
[34] S.D. Conner, S.L. Schmid, Regulated portals of entry into the cell, Nature 422 (2003) 37–44.
[35] J. Zhong, B. Guo, J. Xie, S. Deng, N. Fu, S. Lin, G. Li, Y. Lin, X. Cai, Crosstalk between adipose-derived stem cells and chondrocytes: when growth factors matter, Bone Res. 4 (2016), 15036.
[36] J.A. Swanson, C. Watts, Macropinocytosis, Trends Cell Biol. 5 (1995) 424–428.
[37] S. Kumari, M.G. Swetha, S. Mayor, Endocytosis unplugged: multiple ways to enter the cell, Cell Res. 20 (2010) 256–275.
[38] N. Hao, L. Li, Q. Zhang, X. Huang, X. Meng, Y. Zhang, D. Chen, F. Tang, L. Li, The shape effect of PEGylated mesoporous silica nanoparticles on cellular uptake pathway in Hela cells, Microporous Mesoporous Mater. 162 (2012) 14–23.
[39] P. Kolhar, A.C. Anselmo, V. Gupta, K. Pant, B. Prabhakarpandian, E. Ruoslahti, S. Mitragotri, Using shape effects to target nanoparticles to lung and brain endothelium, PNAS 110 (2013) 10753–10758.
[40] M. Howard, B.J. Zern, A.C. Anselmo, V.V. Shuvaev, S. Mitragotri, Vascular targeting of nanocarriers: perplexing aspects of the seemingly straightforward paradigm, ACS Nano 8 (2014) 4100–4132.
[41] A.C. Anselmo, V. Gupta, B. Zern, D. Pan, M. Zakrewsky, V. Muzykantov, S. Mitragotri, Delivering nanoparticles to lungs while avoiding liver and spleen through adsorption on red blood cells, ACS Nano 7 (2013) 11129–11137.

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[42] A. Loureiro, J. Noro, A.S. Abreu, E. Nogueira, D. Soares Da Costa, C. Silva, A. Cavaco-Paulo, Absence of albumin improves in vitro cellular uptake and disruption of poloxamer 407-based nanoparticles inside cancer cells, Mol. Pharm. 15 (2018) 527–535.
[43] P. Liu, X. Rong, J. Laru, B. van Veen, J. Kiesvaara, J. Hirvonen, T. Laaksonen, L. Peltonen, Nanosuspensions of poorly soluble drugs: preparation and development by wet milling, Int. J. Pharm. 411 (2011) 215–222.
[44] T.S. Levchenko, R. Rammohan, A.N. Lukyanov, K.R. Whiteman, V.P. Torchilin, Liposome clearance in mice: the effect of a separate and combined presence of surface charge and polymer coating, Int. J. Pharm. 240 (2002) 95–102.
[45] J.S. Zhang, F. Liu, F. Huang, Implications of pharmacokinetic behavior of lipoplex for its inflammatory toxicity, Adv. Drug Deliv. Rev. 57 (2005) (2005) 689–698.
[46] F. Madani, S. Lindberg, U. Langel, S. Futaki, A. Graslund, Mechanisms of cellular uptake of cell-penetrating peptides, J. Biophys. 2011 (2011), 414729.
[47] N. Papo, M. Shahar, L. Eisenbach, Y. Shai, A novel lytic peptide composed of DL-amino acids selectively kills cancer cells in culture and in mice, J. Biol. Chem. 278 (2003) 21018–21023.
[48] Y. Lu, Y. Li, W. Wu, Injected nanocrystals for targeted drug delivery, Acta Pharm. Sin. B 6 (2016) 106–113.
[49] T. Li, Y. Lei, M. Guo, H. Yan, Crosslinked poly(vinyl alcohol) hydrogel microspheres containing dispersed fenofibrate nanocrystals as an oral sustained delivery system, Eur. Polym. J. 101 (2018) 77–82.
[50] L. Gao, D. Zhang, M. Chen, C. Duan, W. Dai, L. Jia, W. Zhao, Studies on pharmacokinetics and tissue distribution of oridonin nanosuspensions, Int. J. Pharm. 355 (2008) 321–327.
[51] N. Darville, M. van Heerden, A. Vynckier, M. De Meulder, P. Sterkens, P. Annaert, G. Van den Mooter, Intramuscular administration of paliperidone palmitate extended-release injectable microsuspension induces a subclinical inflammatory reaction modulating the pharmacokinetics in rats, J. Pharm. Sci. 103 (2014) 2072–2087.
[52] N. Darville, M. van Heerden, T. Erkens, S. De Jonghe, A. Vynckier, M. De Meulder, A. Vermeulen, P. Sterkens, P. Annaert, G. Van den Mooter, Modeling the time course of the tissue responses to intramuscular long-acting paliperidone palmitate nano −/microcrystals and polystyrene microspheres in the rat, Toxicol. Pathol. 44 (2016) 189–210.
[53] A. Sarnes, M. Kovalainen, M.R. Häkkinen, T. Laaksonen, J. Laru, J. Kiesvaara, O. Ilkka, S. Oksala, K. Rönkkö, J. Järvinen, L. Peltonen Hirvonen, Nanocrystal-based per-oral itraconazole delivery: superior in vitro dissolution enhancement versus Sporanox® is not realized in in vivo drug absorption, J. Control. Release 180 (2014) 109–116.
[54] A. Tuomela, P. Liu, J. Puranen, S. Rönkkö, T. Laaksonen, G. Kalesnykas, O. Oksala, J. Ilkka, J. Laru, K. Järvinen, J. Hirvonen, L. Peltonen, Brinzolamide nanocrystal formulations for ophthalmic delivery: reduction of elevated intraocular pressure in vivo, Int. J. Pharm. 467 (2014) 34–41.
[55] J. Li, Y. Qiao, Z. Wu, Nanosystem trends in drug delivery using quality-by-design concept, J. Control. Release 256 (2017) 9–18.
[56] L. Peltonen, C. Strachan, Understanding critical quality attributes for nanocrystals from preparation to delivery, Molecules 20 (2015) 22286–22300.
[57] N.P. Truong, M.R. Whittaker, C.W. Mak, T.P. Davis, The importance of nanoparticle shape in cancer drug delivery, Expert Opin. Drug Deliv. 12 (2014) 129–142.
[58] J. Zhao, H. Lu, S. Wong, M. Lu, P. Xiao, M.H. Stenzel, Influence of nanoparticle shapes on cellular uptake of paclitaxel loaded nanoparticles in 2D and 3D cancer models, Polym. Chem. 8 (2017) 3317–3326.
[59] A. Tuomela, J. Hirvonen, L. Peltonen, Stabilizing agents for drug nanocrystals: effect on bioavailability, Pharmaceutics 8 (2016) 16.
[60] M. Kaasalainen, V. Aseyev, E. von Haartman, D. Sen Karam, E. Mäkilä, H. Tenhu, J. Rosenholm, J. Salonen, Size, stability, and porosity of mesoporous nanoparticles characterized with light scattering, Nanoscale Res. Lett. 12 (2017) 74.
[61] B. Van Eerdenbrugh, B. Stuyven, L. Froyen, J. Van Humbeeck, J.A. Martens, P. Augustijns, G. Van den Mooter, Downscaling drug nanosuspension production: processing aspects and physicochemical characterization, AAPS PharmSciTech 10 (2009) 44–53.
[62] C.M. Keck, Particle size analysis of nanocrystals. Improved analysis method, Int. J. Pharm. 390 (2010) 3–12.
[63] S. Aleandri, M. Schönenberger, A. Niederquell, M. Kuentz, Temperature-induced surface effects on drug nanosuspensions, Pharm. Res. (2018)https://doi.org/10.1007/s11095-017-2300-6.
[64] M. van Sebille, L.J.P. van der Maaten, L. Xie, K. Jarolimek, R. Santbergen, R.A.C.M.M. van Swaaij, K. Leifer, M. Zeman, Nanocrystal size distribution analysis form transmission electron microscopy images, Nano 7 (2015) 20593–20606.
[65] D. Spitzer, B. Risse, F. Schnell, V. Pichot, M. Klaumünzer, M.R. Schaefer, Continuous engineering of nano-cocrystals for medical and energetic applications, Sci. Rep. 4 (2014) 6575.
[66] S.A. Surwase, J.P. Boetker, D. Saville, B.J. Boyd, K.C. Gordon, L. Peltonen, C.J. Strachan, Indomethacin: new polymorphs of an old drug, Mol. Pharm. 10 (2013) 4472–4480.
[67] P.T. Mah, T. Laaksonen, T. Rades, L. Peltonen, C.J. Strachan, Differential scanning calorimetry predicts the critical quality attributes of amorphous glibenclamide, Eur. J. Pharm. Sci. 80 (2015) 74–81.
[68] P. Sharma, W.A. Denny, S. Garg, Effect of wet milling process on the solid state of indomethacin and simvastatin, Int. J. Pharm. 380 (2009) 40–48.
[69] M. Wang, G.C. Rutledge, A.S. Myerson, B.L. Trout, Production and characterization of carbamazepine nanocrystals by electrospraying for continuous pharmaceutical manufacturing, J. Pharm. Sci. 101 (2012) 1178–1188.
[70] J. Aaltonen, P. Heinänen, L. Peltonen, H. Kortejärvi, V.P. Tanninen, L. Christiansen, J. Hirvonen, J. Yliruusi, J. Rantanen, In situ measurement of solvent-mediated phase transformations during dissolution testing, J. Pharm. Sci. 95 (2006) 2730–2737.
[71] R. Pireddu, C. Sinico, G. Ennas, F. Marongiu, R. Muzzalupo, F. Lai, A.M. Fadda, Novel nanosized formulations of two diclofenac acid polymorphs to improve topical bioavailability, Eur. J. Pharm. Sci. 77 (2015) 208–215.
[72] H. Eerikäinen, L. Peltonen, J. Raula, J. Hirvonen, E.I. Kauppinen, Nanoparticles containing ketoprofen and acrylic polymers prepared by an aerosol flow reactor method, AAPS PharmSciTech 5 (2004), 68.
[73] F.G. Vogt, J.S. Clawson, M. Strohmeier, A.J. Edwards, T.N. Pham, S.A. Watson, Solid-state NMR analysis of organic cocrystals and complexes, Cryst. Growth Des. 9 (2009) 921–937.
[74] E. Pindelska, A. Sokal, L. Szeleszczuk, D.M. Pisklak, W. Kolodziejski, Solid-state NMR studies of theophylline co-crystals with dicarboxylic acids, J. Pharm. Biomed. Anal. 100 (2014) 322–328.
[75] A. Sokal, E. Pindelska, L. Szeleszczuk, W. Kolodziejski, Pharmaceutical properties of two ethenzamide-gentisic acid cocrystal polymorps: drug release profiles, spectroscopic studies and theoretical calculations, Int. J. Pharm. 522 (2017) 80–89.
[76] V.S. Mandala, S.J. Loewus, M.A. Mehta, Monitoring cocrystal formation via in situ solid-state NMR, J. Phys. Chem. Lett. 5 (2014) 3340–3344.
[77] D. Walsh, D.R. Serrano, Z.A. Worku, B.A. Norris, A.M. Healy, Production of cocrystals in an excipient matrix by spray drying, Int. J. Pharm. 536 (2018) 467–477.
[78] C.B. Aakeröy, D.J. Salmon, M.M. Smith, J. Desper, Cyanophenyloximes: reliable and versatile tools for hydrogen-bond directed supramolecular synthesis of cocrystals, Cryst. Growth Des. 6 (2006) 1033–1042.
[79] S. Kumar, D.J. Burgess, Wet milling induced physical and chemical instabilities of naproxen nano-crystalline suspensions, Int. J. Pharm. 466 (2014) 223–232.
[80] S. Mu, M. Li, M. Guo, W. Yang, Y. Wang, J. Li, Q. Fu, Z. He, Spironolactone nanocrystals for oral administration: different pharmacokinetic performances induced by stabilizers, Colloids Surf. B: Biointerfaces 147 (2016) 73–80.
[81] F. Lai, E. Pini, F. Corrias, J. Perricci, M. Manconi, A.M. Fadda, C. Sinico, Formulation strategy and evaluation of nanocrystal piroxicam orally disintegrating tablets manufacturing by freeze-drying, Int. J. Pharm. 467 (2014) 27–33.
[82] P. Kocbek, S. Baumgartner, J. Kristl, Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs, Int. J. Pharm. 312 (2006) 179–186.
[83] Y. Guinet, L. Paccou, F. Danède, P. Derollez, A. Hédoux, Structural description of the marketed from of valsartan: a crystalline mesophase characterized by nanocrystals and conformational disorder, Int. J. Pharm. 526 (2017) 209–216.
[84] B. Zheng, Z. Zheng, J. Zhang, Q. Liu, J. Wang, X. Luo, L. Wang, Degradation kinetics and by-products of naproxen in aqueous solutions by gamma irradiation, Environ. Eng. Sci. 29 (2012) 386–391.
[85] A.A. Badawi, M.A. El-Nabarawi, D.A. El-Setouhy, S.A. Alsammit, Formulation and stability testing of itraconazole crystalline nanoparticles, AAPS PharmSciTech 12 (2011) 811–820.
[86] S. Kenth, J.-P. Sylvestre, F. Fuhrmann, M. Meunier, J.-C. Leroux, Fabrication of paclitaxel nanocrystals by femtosecond laser ablation and fragmentation, J. Pharm. Sci. 100 (2011) 1022–1030.
[87] Y. Gao, H. Zu, J. Zhang, Enhanced dissolution and stability of adefovir dipivoxil by cocrystal formation, J. Pharm. Pharmacol. 63 (2011) 483–490.
[88] S. D'Souza, A review of in vitro drug release test methods for nano-sized dosage forms, Adv. Pharm. 2014 (2014), 304757.
[89] P.P. Ige, R.K. Baria, S.G. Gattani, Fabrication of fenofibrate nanocrystals by probe sonication method for enhancement of dissolution rate and oral bioavailability, Colloids Surf. B: Biointerfaces 108 (2013) 366–373.
[90] H. Yamashita, C.C. Sun, Improving dissolution rate of carbamazepine-glutaric acid cocrystal through solubilization by excess coformer, Pharm. Res. 35 (2018) 4.
[91] S.L. Childs, P. Kandi, S.R. Lingireddy, Formulation of a danazol cocrystal with controlled supersaturation plays an essential role in improving bioavailability, Mol. Pharm. 10 (2013) 3112–3127.
[92] S.A. Surwase, L. Itkonen, J. Aaltonen, D. Saville, T. Rades, L. Peltonen, C.J. Strachan, Polymer incorporation method affects the physical stability of amorphous indomethacin in aqueous suspension, Eur. J. Pharm. Biopharm. 96 (2015) 32–43.
[93] M. Ullah, I. Hussain, C.C. Sun, The development of carbamazepinesuccinic acid cocrystal tablet formulations with improved in vitro and in vivo performance, Drug Dev. Ind. Pharm. 42 (2016) 969–976.
[94] C.G. Wang, Q. Tong, X.L. Hou, Y. Hu, J.G. Fang, C.C. Sun, Enhancing bioavailability of dihydromyricetin through inhibiting precipitation of soluble cocrystals by a crystallization inhibitor, Cryst. Growth Des. 16 (2016) 5030–5039.
[95] S.J. Nehm, B. Rodriguez-Spong, N. Rodriguez-Hornedo, Phase solubility diagrams of cocrystals are explained by solubility product and solution complexation, Cryst. Growth Des. 6 (2006) 592–600.
[96] H. Yamashita, C.C. Sun, Harvesting potential dissolution advantages of soluble cocrystals by depressing precipitation using the common coformer effect, Cryst. Growth Des. 16 (2016) 6719–6721.
[97] P. Liu, O. De Wulf, J. Laru, T. Heikkilä, B. van Veen, J. Kiesvaara, J. Hirvonen, L. Peltonen, T. Laaksonen, Dissolution studies of poorly soluble drug nanosuspensions in non-sink conditions, AAPS PharmSciTech 14 (2013) 748–756.
[98] D.D. Sun, H. Wen, L.S. Taylor, Non-sink dissolution conditions for predicting product quality and in vivo performance of supersaturating drug delivery systems, J. Pharm. Sci. 105 (2016) 2477–2488.
[99] M. Pellett, S. Castellano, J. Hadgraft, A. Davis, The penetration of supersaturated solutions of piroxicam across silicone membranes and human skin in vitro, J. Control. Release 46 (1997) 205–214.
[100] J.M. Miller, A. Beig, R.A. Carr, J.K. Spence, A. Dahan, A winewin solution in oral delivery of lipophilic drugs: supersaturation via amorphous solid dispersions increases apparent solubility without sacrifice of intestinal membrane permeability, Mol. Pharm. 9 (2012) 2009–2016.

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[101] S.A. Raina, G.G.Z. Zhang, D.E. Alonzo, J. Wu, D. Zhu, N.D. Catron, Y. Gao, L.S. Taylor, Enhancements and limits in drug membrane transport using supersaturated solutions of poorly water soluble drugs, J. Pharm. Sci. 103 (2014) 2736–2748.
[102] J.L. Terebetski, B. Michniak-Kohn, Combining ibuprofen sodium with cellulosic polymers: a deep dive into mechanisms of prolonged supersaturation, Int. J. Pharm. 475 (2014) 536–546.
[103] K. Ueda, K. Higashi, K. Yamamoto, K. Moribe, In situ molecular elucidation of drug supersaturation achieved by nano-sizing and amorphization of poorly water-soluble drug, Eur. J. Pharm. Sci. 77 (2015) 79–89.
[104] K. Shiraki, N. Takata, R. Takano, Y. Hayashi, K. Terada, Dissolution improvement and the mechanism of the improvement from cocrystallization of poorly water-soluble compounds, Pharm. Res. 25 (2008) 2581–2592.
[105] A. Sarnes, J. Østergaard, S. Smedegaard Jensen, J. Aaltonen, J. Rantanen, J. Hirvonen, L. Peltonen, Dissolution study of nanocrystal powders of a poorly soluble drug by UV imaging and channel flow methods, Eur. J. Pharm. Sci. 50 (2013) 511–519.
[106] M. McAllister, Dynamic dissolution: a step closer to predictive dissolution testing? Mol. Pharm. 7 (2010) 1374–1387.
[107] A. Dokoumetzidis, P. Macheras, A century of dissolution research: from Noyes and Whitney to the biopharmaceutics classification system, Int. J. Pharm. 321 (2006) 1–11.
[108] S.G. Kazarian, J. van der Weerd, Simultaneous FTIR spectroscopic imaging and visible photography to monitor tablet dissolution and drug release, Pharm. Res. 25 (2008) 853–860.
[109] J. Kowalczuk, J. Tritt-Goc, A possible application of magnetic resonance imaging for pharmaceutical research, Eur. J. Pharm. Biopharm. 42 (2011) 354–364.
[110] H. Metz, K. Mader, Benchtop-NMR and MRI—a new analytical tool in drug delivery research, Int. J. Pharm. 364 (2008) 170–175.
[111] K.P. Nott, Magnetic resonance imaging of tablet dissolution, Eur. J. Pharm. Biopharm. 74 (2010) 78–83.
[112] J. Østergaard, E. Meng-Lund, S.W. Larsen, C. Larsen, K. Petersson, J. Lenke, H. Jensen, Real-time UV imaging of nicotine release from transdermal patch, Pharm. Res. (12) (2010) 2614–2623.
[113] J. van der Weerd, S.G. Kazarian, Release of poorly soluble drugs from HPMC tablets studied by FTIR imaging and flow-through dissolution tests, J. Pharm. Sci. 94 (2005) 2096–2109.
[114] P. Kayaert, B. Li, I. Jimidar, F. Rombaut, F. Ahssini, G. Van den Mooter, Solution calorimetry as an alternative approach for dissolution testing of nanosuspensions, Eur. J. Pharm. Biopharm. 76 (2010) 507–513.
[115] P. Liu, T. Viitala, A. Kartal-Hodzig, H. Liang, T. Laaksonen, J. Hirvonen, L. Peltonen, Interaction studies between indomethacin nanocrystals and PEO/PPO copolymer stabilizers, Pharm. Res. 32 (2015) 628–639.
[116] Y. Zambito, E. Pedreschi, G. Di Colo, Is dialysis a reliable method for studying drug release from nanparticulate systems? – A case study, Int. J. Pharm. 434 (2012) 28–34.
[117] L. Xie, S. Beyer, V. Vogel, M.G. Wacker, W. Mäntele, Assessing the drug release from nanoparticles: overcoming the shortcomings of dialysis by using novel optical techniques and a mathematical model, Int. J. Pharm. 488 (2015) 108–119.
[118] G. Moreno-Bautista, K.C. Tam, Evaluation of dialysis membrane process for quantifying the in vitro drug-release from colloidal drug carriers, Colloids Surf. A Physicochem. Eng. Asp. 389 (2011) 299–303.
[119] S.K. Brar, M. Verma, Measurement of nanoparticles by light-scattering techniques, Trends Anal. Chem. 30 (2011) 4–17.

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